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Magnetic Resonance Perfusion and Cerebrovascular Studies in Sickle Cell Disease

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Abstract

Sickle cell disease (SCD) is a group of inherited haemoglobinopathies caused by a mutation resulting in an abnormal haemoglobin (HbS). Sickle cell anaemia is the homozygous state (HbSS). Under deoxygenated conditions, the red cell acquires an elongated 'sickle' shape in association with vaso-occlusive events and haemolysis. Cerebrovascular disease (CVD) is a serious complication of SCD, which is one of the commonest causes of childhood stroke. Stroke occurs in up 8% of young patients with SCD with an additional 25% having silent infarction on magnetic resonance imaging (MRI). Recurrent stroke occurs in up 67% of patients without regular blood transfusion, the currently recommended treatment.

There are few data on the natural history of CVD in SCD or abnormality in cerebral perfusion of these patients in relation to the clinical presentation. In addition, there has been a lack of scientific evaluation of the physiological effects of blood transfusion for patients with SCD and neurological complications and its effects on cerebral perfusion and CVD.

The research described in this thesis investigates, in both cross-sectional and longitudinal studies, the association of neurological events (coma, stroke, transient ischaemic attacks, seizures, headaches) with perfusion abnormality and progression of CVD in patients with SCD, and the effect of short- and long-term blood transfusion. This research has used MRI, MRA, and perfusion MRI (dynamic susceptibility contrast MRI), transcranial Doppler (TCD) ultrasound, and clinical, haematological, and oxygen saturation data.

The focus has been on studying cerebral perfusion abnormality in different neurological symptoms in patients with SCD and comparing perfusion MRI with other neuroimaging techniques; on investigating factors involved in the progression of CVD and perfusion abnormality over time; and on identifying predictors of recurrent neurological symptoms in this population. In addition, the effect of blood transfusion on cerebral perfusion and CVD is described and discussed.

This PhD thesis is dedicated,

In memory of my dear parents, Architect Efraim Israel Prengler and Psychologist Martha Scheinker Prengler, who gave me so much love, strength and inspiration.

To my dear sister Paula who is away, but so near to me.

To my aunt Edith and my aunt Rebeca for giving me their caring and family love.

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To my dear friend Professor Steven G. Pavlakis, Professor of Paediatric Neurology in the United States, who gave me a new vision of Medicine as a science.

...And to Graham, for his love and support.

In 1995, Weatherall wrote that sickle cell disease was the first human disease to be defined on a molecular level. Despite this understanding, the relationship between genotype and phenotype remained elusive. Weatherall stated, "We seem a long way from being able to predict the clinical course and outlook of an individual patient."

Professor Steven G. Pavlakis, Child Neurologist, New York, U.S.A.

From 'Sickle Cell Disease: The Neurological Complications.' Mara Prengler, Steven G. Pavlakis, Isak Prohovnik and Robert J. Adams; Annals of Neurology 2002; 51:543-552.
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'Dios mueve al jugador, y éste la pieza.
¿Qué Dios detrás de Dios la trama empieza?'

'God moves the player, and the player the piece.
Which God behind God the weft starts?'

Jorge Luis Borges, Argentinian Poet and Writer

From 'La tabla de Flandes' / 'The Flanders Panel', by Arturo Pérez-Reverte

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Publications Arising from this Thesis

Chapters in Books

1. Prengler M, Hogan A, Kirkham FJ. Sickle cell disease. Stroke and cerebrovascular disease in childhood. ICNA series of monographs in Child Neurology, MacKeith Press 2003; in press.

2. Kirkham FJ and Prengler M. Genetics of paediatric stroke. In Markus H. Genetics of Stroke. Oxford University Press 2003, pp 283-305.

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1. Prengler M, Pavlakis S, Prohovnik I, Adams RJ. Sickle cell disease: The Neurological Complications. Annals of Neurology 2002; 51: 543-552. IF 8.48

2. Prengler M, Pavlakis S, Prohovnik I, Adams RJ. Sickle cell disease: The Neurological Complications. Reply letter (Peripheral Nervous System Complications of Sickle Cell Disease). Annals of Neurology 2003; 53: 143-144.

3. Zafeiriou DI, Prengler M, Gombakis N, Kouskouras K, Economou M, Kardoulas A, Tsantali C, Dimitriadis A, Kirkham F, Athanasiou M. Central nervous system abnormalities in asymptomatic children and adolescents with s/b-thalassaemia. Annals of Neurology 2004; 55: 835-839.

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1. Prengler M, Cox TC, Klein N, Evans JPM, Bynevelt M, Chong WK, Kirkham FJ. Progressive cerebrovascular disease in childhood stroke: associations and effect on recurrence risk. Dev Med Child Neurol 2000; 42 suppl 85: 47. (Poster presented at the British Paediatric Neurology Association conference, December 2000).

2. Prengler M, Boyd S, Kirkham FJ. Seizures in sickle cell disease. Epilepsia 2001; 42 (supp 2): 165. (Poster presented at the 24th International Epilepsy Congress, Buenos Aires, May 2001).

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4. Prengler M, Ganesan V, Dick M, Pohl K, Evans JP, Robbins A, Rossiter M, Yardumian A, Parker N, McEnery G, Telfer P, Cox TC, Chong WK, Kirkham FJ. Risk factors for recurrent transient ischaemic event and stroke in sickle cell disease Dev Med Child Neurol 2001; 43 suppl 90: 27. (Poster presentation at the British Paediatric Neurology Association meeting, Newcastle, Jan 2002).

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7. Prengler M, Calamante F, Saunders D, Perthen J, Chong WK, Evans J, Yardumian A, Telfer P, Davies S, Robins A, Connelly A, Kirkham FJ. Cerebral perfusion, cerebrovascular disease, and haematology in sickle cell disease: A longitudinal study. Ann Neurol, 2003, **54** (suppl 7); S 138. Poster presentation at the Child Neurology Society, Miami, October 2003.

8. Prengler M, Calamante F, Saunders D, Perthen J, Chong WK, Evans J, Yardumian A, Telfer P, Davies S, Robins A, Connelly A, Kirkham FJ. Cerebral perfusion, cerebrovascular disease, and haematology in sickle cell disease: A longitudinal study. European Journal of Paediatric Neurology, **7** (5); 269. Poster presentation at the European Paediatric Neurology Society, Taormina, October 2003.

9. Hogan A, Prengler M, Kirkham F, Telfer P, Vargha-Khadem F, deHaan M. Neurodevelopmental delay in infants with sickle cell disease. European Journal of Paediatric Neurology, **7** (5); 312. Poster presentation at the European Paediatric Neurology Society, Taormina, October 2003.

10. Prengler M, Calamante F, Connelly A, Saunders D, Chong WK, Davies S, Yardumian A, Kirkham FJ. Blood transfusion in sickle cell disease does not necessarily improve cerebral perfusion in the short or long term. Dev Med Child Neurol 2004, **46** (suppl 98): 10. Poster with oral presentation at the British Paediatric Neurology Association (BPNA) 2004 Conference, Sheffield, January 2004.

Glossary

The following abbreviations appear in this thesis (by alphabetical order):

A1 = *Horizontal segment of the anterior cerebral artery*

A2 = *Post-communicating segment of the anterior cerebral artery*

ACA = *Anterior cerebral artery*

ADC = *Apparent diffusion coefficient*

AIF = *Arterial input function*

ASL = *Arterial spin labelling*

BAT = *Bolus arrival time*

BMT = *Bone marrow transplant*

BOLD = *Blood Oxygen Level Dependent MRI*

BTx = *Blood transfusion*

CBF = *Cerebral blood flow*

CBV = *Cerebral blood volume*

CC = *Correlation coefficient (Spearman's rank test)*

CNS = *Central nervous system*

CPAP = *Continuous positive airway pressure*

CSSCD = *Cooperative Study of Sickle Cell Disease (U.S.A.)*

CVD = *Cerebrovascular disease*

DP or DBP = *Diastolic blood pressure*

DSC-MRI = *Dynamic susceptibility contrast MRI or 'bolus tracking' (Perfusion MRI using intravenous Gadolinium diethylenetriaminepenta-acetic acid as contrast agent)*

DTPA = *diethylenetriaminepenta-acetic acid (Gadolinium-DTPA)*

DWI = *Diffusion weighted imaging*

EEG = *Electroencephalogram*

EPI = *Echo-planar imaging*

Gd = *Gadolinium (Gadolinium-DTPA)*

Hb = *Haemoglobin*

HbF = *Foetal haemoglobin*

HbSS = *Homozygous for HbS (sickle cell anaemia)*

ICA = *Internal carotid artery*

IQ = *Intelligence quotient*

L: H ratio = *Lowest: highest ipsilateral maximum middle cerebral artery velocities ratio*

M1 = *Horizontal segment of the middle cerebral artery*

M2 = *Sylvian segment of the middle cerebral artery*

MAP = *Mean arterial blood pressure*

MaxACA = *Maximum averaged anterior cerebral artery velocities (transcranial Doppler ultrasound)*

MaxMCA = *Maximum averaged middle cerebral artery velocities (transcranial Doppler ultrasound)*

MCA = *Middle cerebral artery*

MPC = *Maximum peak concentration (summary parameter)*

MR = *Magnetic resonance*

MRA = *Magnetic resonance angiography*

MRI = *Magnetic resonance imaging*

MTT = *Mean transit time*

NO = *Nitric Oxide*

P1 = *First segment of the posterior cerebral artery*

P2 = *Post-communicating segment of the posterior cerebral artery*

PCA = *Posterior cerebral artery*

PET = *Positron emission tomography*

PLKE = *Posterior leukoencephalopathy*

qT1 MRI = *Quantitative T1 MRI*

RIND = *Reversible ischaemic neurological deficit*

SCA = *Sickle cell anaemia*

SCD = *Sickle cell disease*

SP or SBP = *Systolic blood pressure*

SpO_2 = *Oxygen saturation percentage by pulse oximetry (awake)*

STOP = *Stroke Prevention Study in Sickle Cell Disease (U.S.A.)*

SVD = *Singular value decomposition*

TCD = *Transcranial Doppler ultrasound*

TE = *Echo time*

TIA = *Transient ischaemic attack*

TICA = *Terminal internal carotid artery*

TR = *Repetition time*

TTP = *Time-to- peak (summary parameter)*

VCAM = *Vascular cellular adhesion molecule*

WCC = *White cell count*

WS = *Deep watershed infarct*

Chapter 1: General Introduction: Overview of Sickle Cell Disease

1.1. Genetics and Epidemiology of Sickle Cell Disease

Sickle cell disease (SCD) is a group of inherited haemoglobinopathies (Ballas 1998) common among peoples of Equatorial African ancestry but not confined to that population (Serjeant 1997). The condition is caused by a mutation in the gene for globin, an essential component of the haemoglobin molecule (Hb), which is located inside the red blood cells and carries oxygen to every cell in the body. The pattern of inheritance is autosomal recessive and the homozygous state (HbSS) is known as sickle cell anaemia (Serjeant 1997).

Normal adult haemoglobin (Hb), HbA, consists of two α and two β polypeptides chains ($\alpha_2 \beta_2$). HbA comprises about 97% of the Hb in adults. Two other haemoglobins, Hb A_2 ($\alpha_2 \delta_2$) and HbF ($\alpha_2 \gamma_2$), are found in smaller amounts (1.5 to 3% and less than 1%, respectively) (Murphy 1994). The mutation which produces HbS is the substitution of valine for glutamic acid at the sixth position of the β-globin chain (β6Glut$\rightarrow$Val). Haemoglobin S behaves like normal haemoglobin when fully oxygenated but at low oxygen tension, the haemoglobin S polymerises resulting in gel formation. As a consequence, the red cell has an increased density and elongated sickle shape, becoming less pliable (Pavlakis et al 1989, Serjeant 1997, Alavi 1984) (figure 1.1). The rigid and deformed sickle cell is damaged by mechanical stress during the passage through the vasculature (especially in small blood vessels), resulting in a chronic haemolytic anaemia with red cell destruction (two to eight times higher than normal). The rate of red cell production and destruction in sickle cell anaemia is influenced by infection, drugs and other factors and if the balance is disturbed, anaemic crisis may result (Pavlakis et al 1989).

Figure 1.1. Red blood cell with the characteristic sickle shape (arrow) found in sickle cell anaemia. (From Embury SH, Hebbel RP, Mohandas N, Steinberg MH (eds): *Sickle Cell Disease: Basic Principles and Clinical Practice.* Raven Press, Ltd., New York, 1994)

Other double heterozygote variants of the SCD syndromes include SC disease (inheritance of one HbS with one gene for Hb C), the next in frequency after sickle cell anaemia. Co-inheritance of a β-thalassaemia gene with HbS causes sickle/β^+-thalassaemia with 3-25% HbA, or sickle/β^0-thalassaemia without HbA but often (not always) with increased HbF and normal or slightly reduced HbA2 (Ballas 1998, Serjeant 1997, Zafeiriou et al 2004). Clinically, sickle cell anaemia (HbSS) and sickle/β^0- thalassaemia tend to run a severe course, whereas haemoglobin SC disease and sickle/β^+- thalassaemia have milder manifestations (Serjeant 1997).

Although this gene mutation is most common in equatorial Africa, it is also found in the Mediterranean regions of Europe and Turkey (Ranney 1994). The carrier state of the sickle cell gene (one HbS gene and a normal β globin gene) is defined as sickle cell trait (see Serjeant 1997) and protects against malaria, as sickle cells are a hostile enviroment for the parasite (Pavlakis et al 1989). In SCD, carrier frequency is between 5-40% in the populations where the frequency of the gene is high (Ballas 1998, Serjeant 1997). The phenotype may be influenced by the different β-haplotypes patterns of polymorphisms in the β globin chain. There are three major African and African American haplotypes: Senegal, Benin and Bantu (or Central African Republic) (Serjeant 1997). In

addition, there is an independent haplotype in India and Saudi Arabia (Ballas 1998). The sickle gene is distributed worldwide as a consequence of the slave trade and economic migration (Nagel 1994, figure 1.2).

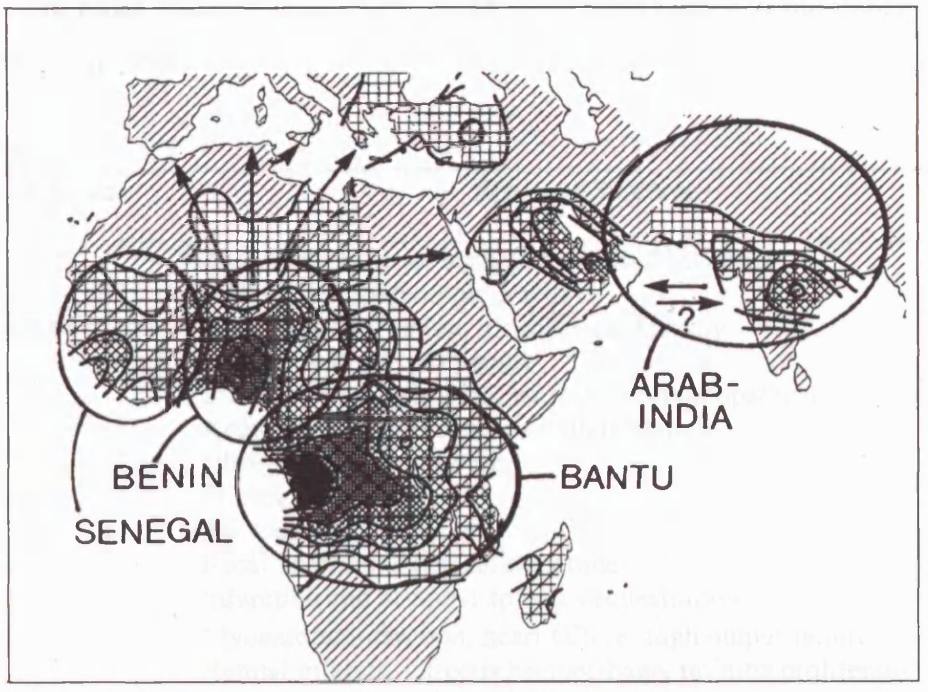


Figure 1.2. Shaded areas of this map represent progressively higher frequencies (in direct proportion to darker intensity) of the β^S gene (Senegal, Benin, Bantu, and Arab-Indian haplotypes). From Nagel RL. Origins and dispersion of the sickle gene, in Embury SH, Hebbel RP, Mohandas N, Steinberg MH (eds): *Sickle Cell Disease: Basic Principles and Clinical Practice.* Raven Press, Ltd., New York, 1994, page 355).

1.2 Non-Neurological Problems of Sickle Cell Disease

There are a large number of complications of sickle cell disease, listed in table 1.1. Patients with SCD present with symptoms secondary to two main mechanisms: haemolytic anaemia and vaso-occlusion (Pavlakis et al 1989, Serjeant 1997). The leading causes of death among very young children with SCD are firstly acute anaemia (caused by splenic sequestration and aplastic crisis due to viral infection) and, secondly bacterial infection, particularly secondary to Pneumoccocal meningitis or pneumonia (as the spleen becomes non-functional) (Platt et al 1994). There is also substantial

morbidity from early infancy due to vaso-occlusive events, which cause painful bone crisis, abdominal pain, acute chest syndrome and neurological events (Pavlakis et al 1989). In the USA, the median age at death for males with sickle cell anaemia (HbSS) is 42 years and for females, 48 years; survival is better for those with HbSC disease (Platt et al 1994). In older children and adults, death occurs secondary to pulmonary or renal failure (Platt et al 1994).

Organ or Tissue	Effect of Sickling
Joints	Aseptic necrosis of head of femur and humerus
Long bones	Infarcts with pathologic fractures
Vertebral body	Compression fracture; biconcave deformity
Lung	Infarcts with cor pulmonale
Brain	Infarcts with hemiplegia; various encephalopathies
Kidney	Acute papillitis necroticans with hematuria
Gut	Ulceration and infarction
Gallbladder	Stones
Skin	Necrosis and ulceration
Liver	Focal necrosis; cirrhosis sometimes
Spleen	Infarction and atrophy; splenic sequestration
Heart	Myocardial infarction; heart failure; high output failure
Eye	Retinal infarcts, vitreous haemorrhage, retinitis proliferans
Adrenal gland	Infarction with Addison's disease rarely
Pancreas	Infarction with diabetes mellitus very rarely
Pituitary	Infarction with hypopituitarism
Breast	Tissue necrosis; fibroadenopathy
Ovary	Infarction, infertility rarely
Penis	Priapism with occasional penile fibrosis and impotence
Arteries	Intermittent claudication; arterial thrombosis
Veins	Phlebothrombosis; dural sinus thrombosis
Nerves	Infarction with asymmetrical motor and sensory neuropathy
Spinal cord	Infarction
Lymph Nodes	Infarction; possible disturbance of immunological status
Uterus	Infarction with ischaemia in pregnancy
Placenta	Multiple infarcts; miscarriages and stillbirths
Bone Marrow	Infarcts with pulmonary embolism (specially in pregnancy)
Teeth	Periodontal tissue infarctions; caries
Whole blood	Massive intravascular sickling with sudden death
Immune system	Complement pathway, phagocytic defects; lymphoid hyperplasia

Table 1.1. Non- Neurological Complications in Sickle Cell Disease: Organ Involvement. *Adapted from Konotey-Ahulu F.D. The sickle cell diseases: Clinical manifestations including the 'sickle cell crises.' *Arch Intern Med* 1974; 133: 611-623 (From Pavlakis et al 1989).

Samuels and colleagues (1992) found a high prevalence of snoring and sleep apnoea, apparently secondary to enlarged tonsils and adenoids, in children with sickle cell disease. In another study, 25% of sickle cell patients were reported to have oxygen saturation levels below 90%; oxygen saturation was lower in patients with acute chest crisis but not with stroke (Homi et al 1997). Hypoxia produces changes in the haemoglobin of the sickle cell leading to increased red and white cell and platelet adhesion (Inwald et al 2000, Setty et al 2003) as well as polymerization, gel formation and potassium efflux associated with increased cell density (Pavlakis et al 1989, Steinberg et al 1999).

1.3. Neurological Complications of Sickle Cell Disease

The WHO definition of stroke is a constellation of clinical symptoms lasting more than 24 hours of vascular aetiology (WHO 1978). Hemiparesis is the typical presentation. In one North American study in which the overall incidence for childhood stroke was 1.29/100,000 per year (ischaemic 0.58/100,000/y, and haemorrhagic 0.71/100,000/y), the commonest cause was SCD (39%), with an incidence of 285/100,000/year (Earley et al 1998), which is similar to that found in general populations of elderly adults. In patients with sickle cell disease, approximately 75% of the infarcts are ischaemic and the remainder are haemorrhagic (Powars et al 1978). The prevalence of clinical stroke in patients with SCD younger than 19 years is 8.1% (Ohene-Frempong 1991) and in addition, the lifetime risk of stroke is between 25 and 30% (Ohene-Frempong 1998, Styles et al 2000).

Patients with prior infarction are at increased risk of haemorrhage as they age (Powars et al 1978). Infarction is common in mid-childhood, between 2 and 10 years of age. Stroke incidence decreases to a minimum between the ages of 20 to 29 years, but there is a further peak after the age of 35. Haemorrhage has the highest incidence in young adults (20-30 years) but is not uncommon in children (Ohene-Frempong 1998, figure 1.3). Recurrence of stroke occurs in up 67% without blood transfusion therapy (Powars et al 1978).

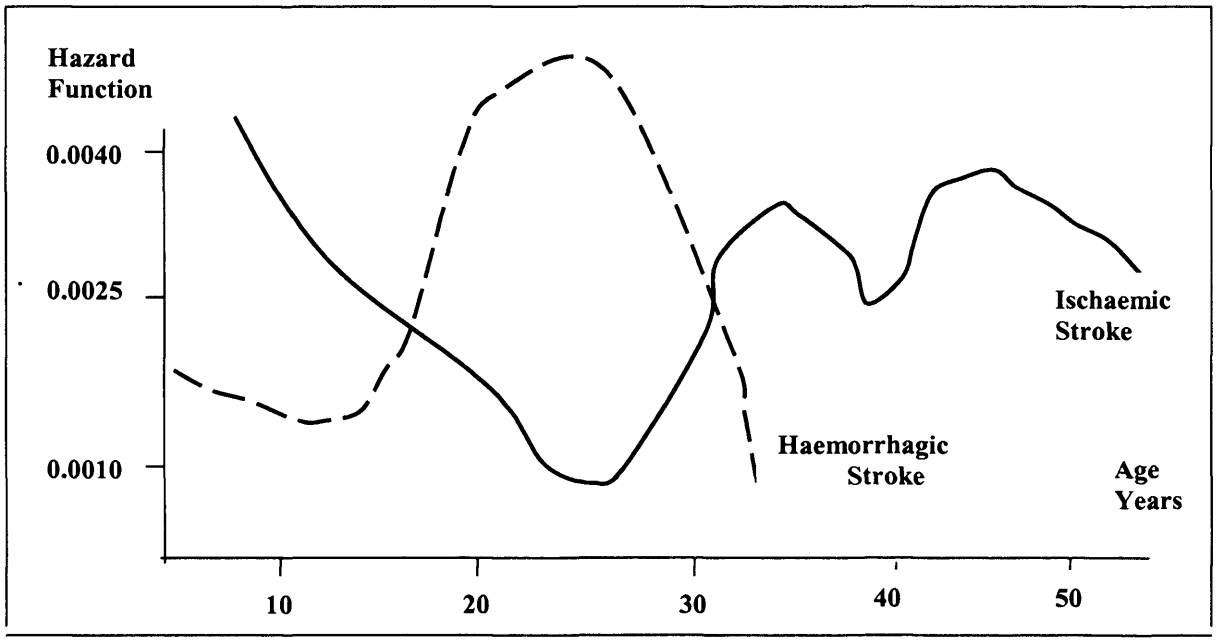


Figure 1.3. Hazard rates of infarctive and haemorrhagic stroke in SS patients by age (adapted from Ohene-Frempong et al 1998).
– Infarctive stroke; -- haemorrhagic stroke

Cerebral infarction may be symptomatic or asymptomatic (silent or covert infarct). Acute neurological symptoms and signs are common in sickle cell disease and, as well as stroke, include headaches, coma, seizures, transient ischaemic accident (TIA), which lasts less than 24 hours, and reversible ischaemic neurological deficit (RIND), which lasts more than 24 hours but recovers completely with no permanent deficit (Adams 1994). Other symptoms of cerebral infarction include dysphasia, difficulty with gait and 'soft neurological signs' (Adams 1994, Mercuri et al 1995). Coma in SCD may be caused by intracranial haemorrhage (Adams 1994), although extensive middle cerebral artery infarction with oedema can also have a similar presentation (Millman et al 1998). In addition, children with SCD can present with central nervous system infections such as meningitis, bacterial abscess and cerebral tuberculoma. The incidence of CNS infections has decreased with penicillin prophylaxis and immunization (Pavlakis et al 1989, Adams 1994). Severe headache can also be a symptom of intracranial

haemorrhage (subdural, intraparenchymal, subarachnoid, or intraventricular) (Adams 1994). Other neurological complications described in sickle cell disease include recurrent seizures, chronic headaches, myelopathy and neuropathy (Pavlakis et al 1989, Adams 1994, Shields et al 1991, Danesi 1983, Liu et al 1994, Prengler et al 2001a, Prengler et al 2002a, Gregory et al 1994, Osontokun 1979). Venous sinus thrombosis and pseudotumor cerebri have also been reported (van Mierlo et al 2003, Henry et al 2004, Pavlakis et al 1989, Adams 1994).

The majority of studies looking at cognitive function in SCD have found a reduction in IQ compared with controls (for review see Schatz et al 2002). Patients with HbSS have lower IQ than those with HbSC, who have lower IQ that those with HbS-thalassaemia (Mashayehki et al 1987). In one study, 33% of young children had an IQ in the range of mild mental retardation (IQ 50-70) (Steen et al 1999a). However, most studies have found IQ to be within the normal range in the majority of patients, but with a mean lower than 100 (Watkins et al 1998, Schatz et al 2002). Performance and full scale IQ are more impaired in patients with sickle cell patients with severe anaemia (haematocrit <20%) and thrombocytosis (> 500 x 10^9/L) (Bernaudin et al 2000). IQ appeared to decrease with age in children followed longitudinally in the US Co-operative Study of Sickle Cell Disease (CSSCD) (Wang et al 2001a). There is therefore clinical evidence that SCD is associated with a chronic encephalopathy that may be progressive (Kugler et al 1993).

1.4. Molecular Sickling

The mechanism of molecular sickling in SCD, which is very important for the physiopathology of the complications, including cerebrovascular disease (CVD), is complex. HbSS behaves like a normal haemoglobin (HbA) when fully oxygenated, but at low oxygen tensions, the haemoglobin S polymerises resulting in gel (Pavlakis et al 1989, Prengler et al 2002b). Gel formation is decreased in the presence of HbA and foetal haemoglobin (HbF) (Pavlakis et al 1989, Franco et al 2000). High concentration of HbSS results in increased sickling with hypoxia, acidosis or infection (Keidan et al 1989, Sultana et al 1998) and the rigid cell may sludge in small blood

vessels (Pavlakis et al 1989). In addition, the endothelial surface may be damaged and become more adhesive to red and white cells and platelets (Pavlakis et al 1989, French et al 1997, Nath et al 2000, Inwald et al 2000, Setty et al 2003), which is a candidate mechanism for endothelial injury and vaso-occlusion in SCD (Sultana et al 1998, Westerman et al 1999, Green et al 1999, Belcher et al 2000, Nath et al 2000, Wajer et al 2000), perhaps resulting in the formation of a nidus of blood cells, and eventually leading to long term arterial narrowing. There is probably an important additional effect of infection (figure 1.4; see section 1.9) and it has been suggested that SCD should be seen as an inflammatory disease of the blood vessels (Belcher et al 2000).

Nitric oxide (NO), synthesised from L-arginine in endothelial cells, is a critical mediator of vascular integrity. NO regulates mainly vasomotor tone and has powerful vasodilatory functions (French et al 1997). Disturbance of endothelial function may reduce NO levels, allowing unopposed vasoconstriction instead of vasodilatation, which may contribute to the genesis of CVD in SCD (French et al 1997).

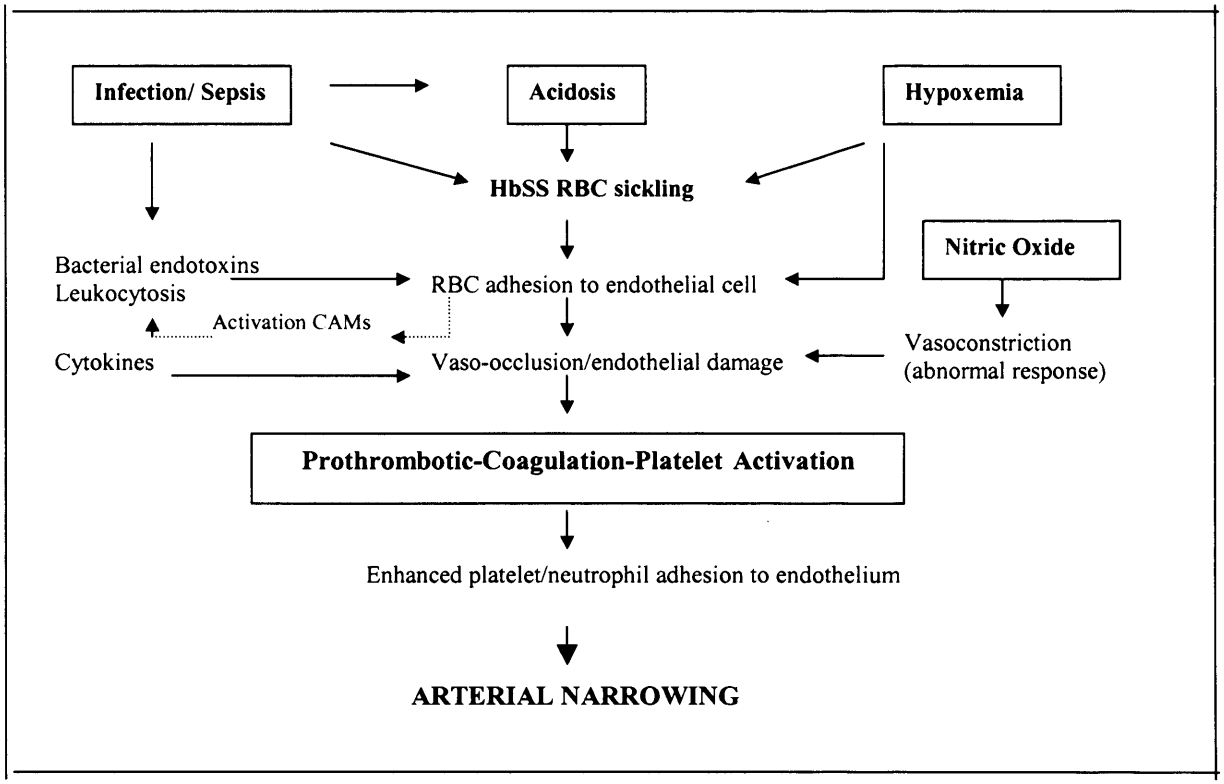


Figure 1.4. Principal molecular, infectious, and prothrombotic mechanisms involved in the genesis of cerebrovascular disease in sickle cell disease. HbSS= sickle cell anaemia; RBC= red blood cell, CAMs= cell adhesion molecules (adapted from Prengler et al 2002b).

1.5. Cerebrovascular Disease in Sickle Cell Disease

1.5.1. Pathology

Endothelial hyperplasia and thrombi in large and small vessels are associated with brain infarcts in autopsied patients with sickle cell disease (Bridgers 1939, Rothman et al 1986, Koshy et al 1990). These data are compatible with those obtained in living patients: for example, in 6 out of 7 patients with stroke, cerebral angiography demonstrated large-artery occlusive disease with intimal narrowing or complete occlusion of the intracranial portion of the internal carotid artery and proximal middle cerebral and anterior cerebral arteries (Stockman et al 1972). Other studies have showed that between 60 and 95% of patients with SCD who underwent angiography had large vessel-disease (Adeloye et al 1970, Stockman et al 1972, Russell et al 1984, Borosh et al 1986).

1.5.2. Mechanism of Small and Large Blood Vessel Disease

There are at least two mechanisms involved in the development of cerebral infarction in SCD. The first mechanism is the large blood vessel disease with endothelial hyperplasia and risk of arterio-arterial thromboembolism. The abnormal red cells and increased blood flow at the level of the cerebral large vessel bifurcation in sickle patients may cause damage secondary to turbulence in the large vessel with thrombus formation and endothelial hyperplasia (Pavlakis et al 1989). The arterial narrowing may cause a relative hypoperfusion in vulnerable regions of the brain (Huttenlocher et al 1984) leading to borderzone infarcts (anterior, posterior or deep watershed (WS) infarcts) in the territory between branches of a central vessel (such as the internal carotid artery [ICA]). The borderzone between two fields is vulnerable if blood pressure decreases. One of the most vulnerable regions of the human brain is the anterior borderzone of the frontal lobe between the territories of the middle and anterior cerebral arteries (Torvik 1984, Rodgers et al 1988). There is a parallel with non-SCD adult patients with large vessel disease (i.e. ICA stenosis), who suffer borderzone infarcts when suffering from acute haemodynamic insufficiency (such as severe heart disease, hypotension or

syncope) (Bogousslavsky et al 1986, Pavlakis et al 1989). In addition, the risk of borderzone stroke in SCD patients may be increased by narrowing of the central large vessel or its branches, which is common in SCD (see section 1.4).

The second likely mechanism is the sludging of the dense sickle cells in the small blood vessels of the brain, such the small distal penetrating arteries of the cortex or lenticulostriates supplying the basal ganglia, although there is little direct evidence. Cerebral blood flow rates and red blood cell oxygen content are lower in these vessels (Kaul et al 1986, Pavlakis et al 1989). These conditions may lead to sickling and vaso-occlusion, which may explain the presence of infarcts in sickle cell patients without large vessel disease (figure 1.5). In any given patient with SCD, either or both mechanisms may play a role in the development of cerebral infarction.

The role of other mechanisms, such as venous sinus thrombosis (Oguz et al 1994, van Mierlo 2003), posterior leukencephalopathy (Coley et al 1999, Henderson et al 2002) demyelination (Lee et al 2002) or acute cerebral oedema, in the development of CNS pathology has not been extensively explored. These pathologies appear to be reversible and may be missed if patients do not receive emergency MR imaging, as exchange transfusion in the local hospital is usually the priority.

As the patient with SCD ages, the progressive narrowing of the large vessel may be associated with aneurysmal dilatation (Berry aneurysms) (Rinkel et al 1993, Diggs et al 1993, Preul et al 1998), or the development of compensatory but fragile collaterals, defined as moyamoya syndrome (Vernet et al 1996, Dobson et al 2002). Both entities may lead to intracranial haemorrhage in young adults, and also in children.

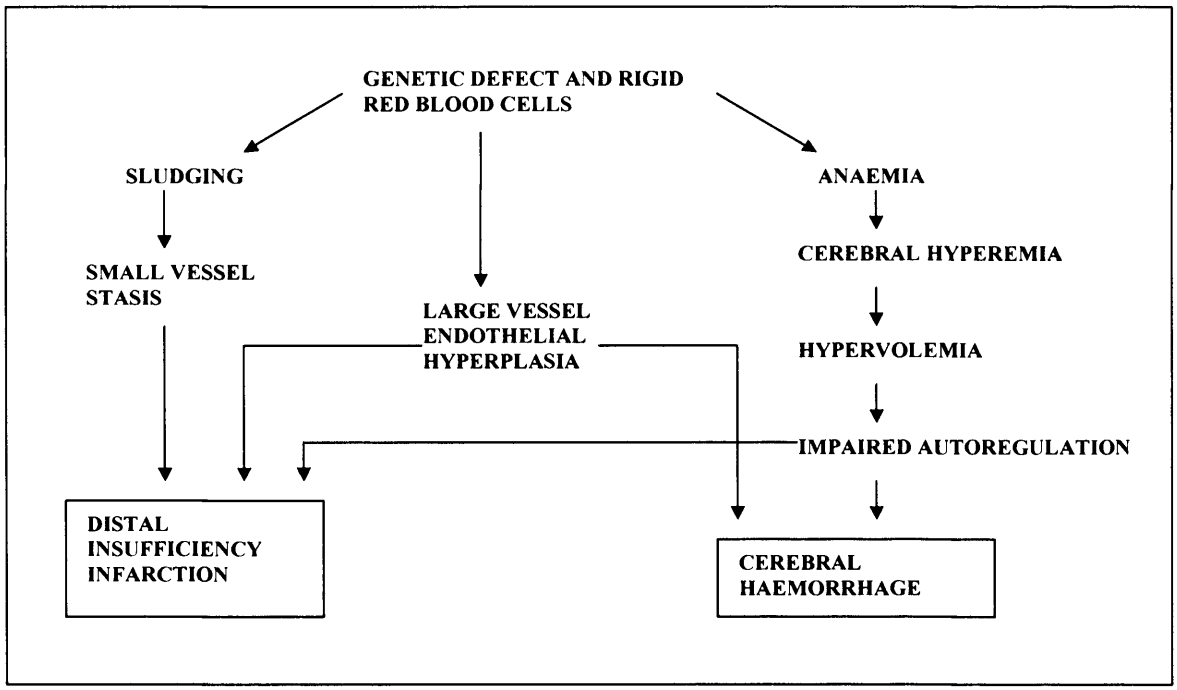


Figure 1.5. Proposed pathophysiology of cerebrovascular disease in SCD (adapted from Pavlakis et al 1989)

1.6. Parenchymal Imaging in Sickle Cell Disease: Stroke and Clinically Silent Disease

1.6.1. Magnetic Resonance Imaging (MRI)

There is a predilection for brain infarction in the borderzone areas of the brain demonstrated in several studies using T2 weighted MRI of the brain (Pavlakis et al 1988, Kugler et al 1993, Adams 1994, Moser et al 1996, figure 1.6). Silent infarction is found in up 25% of sickle cell patients without clinical symptoms (Pavlakis et al 1989, Armstrong et al 1996, Kinney et al 1999, Bernaurdin et al 2000, Miller et al 2001, Saunders et al 2001, Pegelow et al 2002).

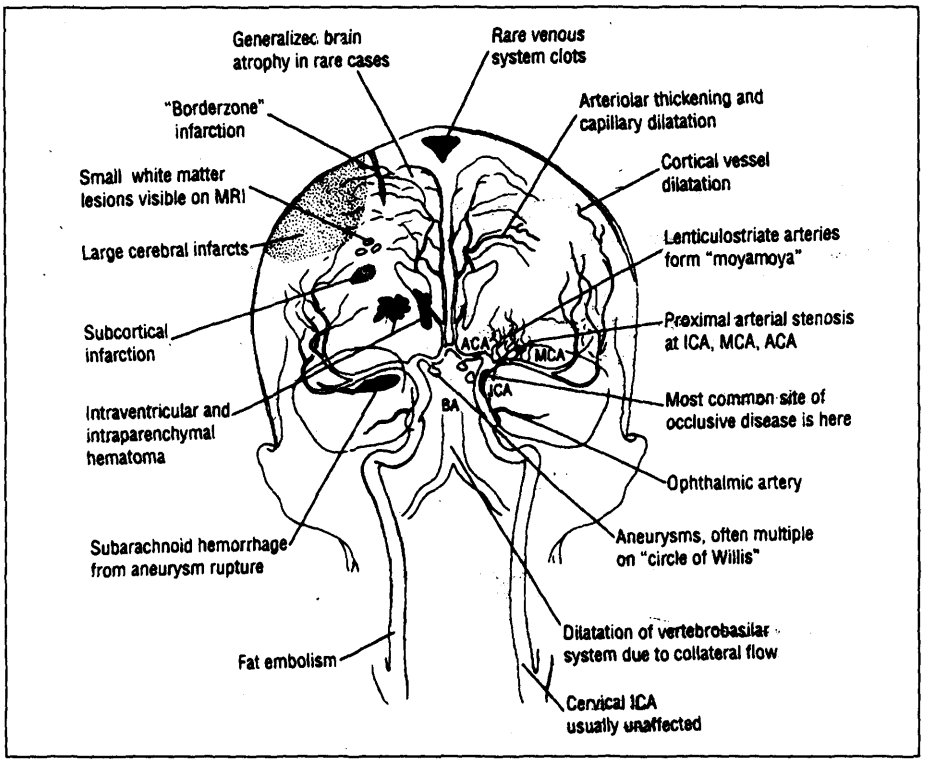


Figure 1.6. The location of the various manifestations of cerebrovascular disease in sickle cell disease (large and small blood vessel disease). 'Borderzone' infarctions are common in the junctional areas between the middle and anterior cerebral and the middle and posterior cerebral arterial distributions. The small white matter lesions visualized on MRI may be seen in otherwise asymptomatic patients. Cerebral infarcts in major vessel occlusion and 'borderzone' patterns are the most common, and fat embolism and venous abnormalities the least common lesions (From Adams RJ. Neurological complications, in Embury SH, Hebbel RP, Mohandas N, Steinberg MH (eds): *Sickle Cell Disease: Basic Principles and Clinical Practice.* Raven Press, Ltd., New York, 1994, page 600).

The high prevalence of silent infarcts on MRI is a distinctive finding in children with SCD compared with children with non-sickle stroke, probably reflecting the chronicity of the vasculopathy in this population. Silent infarction is associated with significant and progressive cerebrovascular disease, such as moyamoya syndrome or cerebral vasculitis, in only in a small proportion of non-sickle stroke in childhood, whereas this is a common pattern in sickle cell disease (Prengler et al 1997).

Patients with SCD and overt or silent infarction are more likely to have abnormal psychometric testing (Armstrong et al 1996, DeBaun et al 1998, Watkins et al 1998). In a study of 194 patients, those with normal MRI scored better on neurodevelopmental testing than those with silent infarction, and patients with silent infarction scored better than those with overt clinical stroke (Armstrong et al 1996). Deficits in executive function and attention (Brown et al 2000), spatial function and memory, are also described, especially in those with anterior lesions or diffuse stroke (Craft et al 1993, Watkins et al 1998). Because of the association with the presence of silent infarcts and progressive encephalopathy, psychometric testing may be a sensitive marker for the extent and progression of clinical and subclinical damage (Pavlakis et al 1989).

Quantitative T1 MRI (qT1 MRI) may add sensitivity to the conventional MRI study. Abnormal qT1 MRI is found in frontal and thalamic regions (Steen et al 1996) in children with SCD as young as four years of age (Steen et al 1999b). This study demonstrated selective damage in the grey rather than in the white matter (Steen et al 1999b). Those with haematocrit lower than 27% (normal haematocrit for non-sickle children range: 34-40% for the same age [Camitta 1996]) and abnormal grey matter were more likely to have abnormal psychometric testing (Steen et al 1999a, Steen et al 1999b). Chronic oxyhaemoglobin desaturation and anaemia in patients with SCD may compromise brain tissue in the absence of CVD.

Cerebral haemorrhage in older patients may be related to the progression of the cerebrovascular disease, leading to aneurysm formation and moyamoya syndrome (Preul et al 1998, Dobson et al 2002) or may occur secondary to venous sinus thrombosis (Gadian et al 2000).

1.7. Cerebral Blood Flow Studies in Sickle Cell Disease

1.7.1. Xenon Inhalation

Studies of *global* cerebral blood flow (CBF) have shown that neurologically asymptomatic patients with sickle cell disease usually have *global* cerebral hyperemia secondary to anaemia and may fail to increase CBF with hypercapnia (Prohnovik et al

1989). Studies with $Xenon^{133}$ demonstrated that cerebral blood flow (CBF) in patients with SCD was 68% greater than in controls (Kugler et al 1993, Prohnovik et al 1989); this increase was inversely related to hematocrit level (Prohnovik et al 1989). This suggests that the increased cerebral blood flow is secondary to adaptive vasodilatation and the vascular reserve capacity may be reduced (Prohnovik et al 1989). However, this hyperaemia may not be seen in patients with acute neurological symptoms. For example, a study using $Xenon^{133}$ demonstrated diffusely decreased perfusion in patients with stupor, coma and seizures (Huttenlocher et al 1984).

1.7.2. Positron Emission Tomography (PET)

Studies of *regional* cerebral blood flow (CBF), for example using positron emission tomography (PET), have shown *focal* changes in CBF in SCD patients in addition: for example an increase in regional CBF (Herold et al 1987); or more extensive regional abnormalities than those shown on MRI (Powars et al 1999). Another PET study showed abnormal regional cerebral metabolic rates for glucose in the frontal lobes of asymptomatic sickle cell patients with normal CT scans (Rodgers et al 1988). There are few data on regional cerebral blood flow in acutely symptomatic patients, but the available studies suggest that, as expected, those with vessel occlusion have an area of reduced CBF distally (Huttenlocher et al 1984).

1.7.3. Perfusion MRI (DSC-MRI)

Perfusion MRI (dynamic susceptibility contrast MRI), using a contrast agent (gadolinium-diethylenetriamine penta-acetic acid), demonstrated perfusion deficits associated with central nervous system (CNS) events (such as stroke, transient ischaemic attack, severe headaches and coma) in symptomatic patients with sickle cell disease (Kirkham et al 2001b). These symptoms persisted in a significant proportion of patients despite transfusion therapy. Patients with transient ischaemic attack had abnormal perfusion when other neuroimaging modalities were normal (Kirkham et al 2001b). Perfusion abnormalities were associated with MRA turbulence, which is a marker of cerebrovascular disease (Tzika et al 1993, Kirkham et al 2001b). Kirkham et al demonstrated that all the patients with severe turbulence or occlusion on MRA had

perfusion abnormalities, but also, there were perfusion abnormalities in some patients with normal MRA and TCD (Kirkham et al 2001b). MRA abnormalities have been associated with abnormal TCD in sickle cell patients with stroke (Seibert et al 1998). TCD velocities higher than 200 cm/sec are associated with perfusion abnormality (Kirkham et al 2001 b) and risk of stroke (Adams et al 1992), but there is also an association between abnormal perfusion and moderately increased TCD velocities (>170 <200 cm/sec) (Kirkham et al 2001b). Another technique to measure cerebral blood flow is *continuous arterial spin-labeling perfusion MR*, which allows quantification of regional cerebral blood flow (CBF) by using magnetically labelled water molecules in arterial blood, without the need of exogenous agents. In a recent study with sickle cell children who had not have acute neurological events and had not received chronic transfusion, Oguz et al demonstrated that mean CBF values were significantly higher in sickle patients than in controls (non-sickle) and, in 4 patients, the baseline CBF was significantly decreased in territories seen as unaffected on conventional MR images and MRA (Oguz et al 2003).

The effect of blood transfusion therapy on cerebrovascular disease and perfusion has not been studied in depth, although of a series of 43 sickle patients on chronic transfusion therapy, 43% had moyamoya collaterals and 41% of the patients experienced recurrent cerebrovascular events despite transfusion (Dobson et al 2002). In one patient with chronic cerebrovascular disease, perfusion abnormalities had improved on a study performed a few days after transfusion compared with the appearances immediately before (Kirkham et al 2001b). Another patient with evidence of bilateral cerebrovascular disease, with high velocities on TCD and turbulence on MRA in association with a widespread perfusion abnormality throughout both hemispheres had completely normal MRI, MRA and perfusion MRI one year later (Kirkham et al 2001b). It is possible that chronic inflammation or infection is associated with progressive CVD and recurrence of CNS events despite a transfusion regime (Belcher et al 2000, Prengler et al 2000b). The effect of blood transfusion on the cerebral vasculature and cerebral perfusion is still uncertain.

1.8. Vascular Studies

1.8.1. Cerebral Angiography

Cerebral angiography has been used in a relatively small proportion of patients with stroke and SCD, because of the risks of inducing crisis with the contrast medium, as well as of stroke. Studies have showed that between 60 and 95% of patients with SCD who underwent angiography had large vessel-disease (Adeloye et al 1970, Stockman et al 1972, Russell et al 1984, Borosh et al 1986). Conventional angiography is the required diagnostic technique to visualise aneurysms (Rinkel et al 1993, Diggs et al 1993, Preul et al 1998).

1.8.2. Magnetic Resonance Angiography

MR angiography (MRA) abnormalities are associated with sub-clinical infarction when flow turbulence involves a long segment (6mm) and there is reduced distal flow (Gilliams et al 1998). In sickle cell disease, MRA can be up to 85% accurate when compared with conventional angiography (Kandeel et al 1996). MRA can detect CVD in very young children, as was shown in a study by Wang et al, where MRA abnormalities were found in 3 out of 29 patients from 7 to 48 months (Wang et al 1998). In patients with SCD with or without cerebral infarction, turbulence on MRA may be graded as mild, moderate and severe, but there are few data looking at the relationship with the degree of arterial stenosis on convèntional angiography (Seibert et al 1998, Kirkham et al 2001b).

1.8.3. Transcranial Doppler Ultrasound

Transcranial Doppler ultrasound (TCD) may be used to measure the mean arterial blood flow velocities. Increased TCD velocities may be associated with a reduced artery diameter, or increased CBF in the presence of anaemia. In sickle cell anaemia, stenosis on conventional arteriography may be detected when the MCA velocities are between 140 and 190 cm/sec; velocities more than 190 cm/sec are associated with marked artery stenosis on angiography (Adams et al 1992). Mean MCA blood flow velocities equal or greater than 200 cm/sec are associated with 40 % of risk of stroke over a three year-period (Adams et al 1997 and 1998). The STOP study has demonstrated that TCD is a

useful tool for screening and detection of patients at risk of stroke in the sickle population. Children with TCD MCA or ICA time-averaged velocities at or over 200 cm/sec are at risk of first stroke in three years (13% per year) if they do not receive blood transfusion therapy (Adams et al 1992, Adams et al 1997, Adams et al 1998). Those with 'conditional' TCD studies, i.e. with velocities between 170 and 200 cm/sec are also at higher risk of stroke than those with velocities <170 cm/sec (Adams et al 2004). Since the trial ended prematurely because there was a very large advantage in favour of blood transfusion, screening and transfusion for those with velocities >200 cm/sec has been recommended as standard care in the USA; recent epidemiological evidence suggests that has been a parallel fall in the incidence of stroke in sickle cell disease (Fullerton et al 2004). TCD may detect cerebrovascular disease (CVD) at an earlier stage than MRA; the highest risk of stroke is in children in whom both are abnormal, whose CVD rarely improves without blood transfusion (Abboud et al 2004) and even then does not normalise completely (Minniti et al 2004). Studies have shown a good correlation between abnormally high TCD velocities and Xenon studies of CBF; hematocrit (Brass et al 1988); conventional angiography (Adams et al 1988); and MRA (Siegel et al 1995, Verlhac et al 1995, Seibert et al 1998). However, there are very few data on the natural history in patients with abnormally low velocities (Minniti et al 2004).

1.9. Risk Factors for Stroke in Sickle Cell Disease

There are several risk factors that predispose individuals with sickle cell disease to develop cerebral infarction. Patients with SC disease and sickle/β^+-thalassaemia are less likely to develop neurological symptoms, while those with homozygous sickle cell anaemia and sickle cell/β^0 thalassaemia have a more severe course with a higher risk of cerebrovascular events (Serjeant 1997, Adams 1994, Ohene-Frempong et al 1998). Age is an important risk factor as the highest incidence of first infarctive stroke is in the first years of life (2-5 years) (Ohene-Frempong 1998).

Patients with clinical stroke have lower steady state haemoglobin concentrations, higher white cell count and higher reticulocyte counts (Balkaran et al 1992, Ohene-Frempong

et al 1998, Miller et al 2000). Children with SCD have a reduction of protein S and C levels but there is little evidence that these prothombotic abnormalities predict stroke (Tam 1997, Liesner et al 1998). Other clinical risk factors for stroke include relative hypertension (Rodgers et al 1993, Pegelow et al 1997), painful crisis, infection, early dactylitis, and other systemic illnesses (Adams 1994, Miller et al 2000, Osuntokun 1979). Nocturnal oxyhaemoglobin desaturation in children with SCD, secondary to upper airway obstruction (Sidman et al 1988, Samuels et al 1992), is associated with stroke and other central nervous system (CNS) events (Robertson et al 1988, Davies et al 1989, Kirkham et al 2001a).

The largest multicenter study to date (the Cooperative Study of Sickle Cell Disease) suggested that the predictors of ischaemic and hemorrhagic clinical stroke and silent infarction might be different. Independent risk factors for ischaemic stroke were previous transient ischaemic attacks, lower steady-state-haemoglobin concentrations, recent acute chest syndrome and higher blood pressure while those for haemorrhagic stroke included a low haemoglobin concentration and a high white cell count (Ohene-Frempong et al 1998). Risk factors for silent infarction were a low pain rate, history of seizures, leukocytosis and the Senegal β^s-globin haplotype (Kinney et al 1999), but the severity of the anaemia was not independently associated.

Infection and chronic inflammation is a recurring theme in the pathophysiology of the neurological complications in sickle cell disease. Evidence of systemic inflammation is very common in sickle cell crisis with increased numbers of activated monocytes, platelets, endothelial cells and adhesion molecules and increased C-reactive protein (Swerlick et al 1993, Belcher et al 2000, Prengler et al 2000, figure 1.4). As well as the evidence that leukocytosis is a risk factor for stroke and silent infarction (Balkaran et al 1992, Ohene-Frempong et al 1998, Kinney et al 1999), cerebrovascular episodes often appear to be precipitated by infections including Pneumococcal meningitis (De Montalembert et al 1993), *Parvovirus* aplastic anaemia (Wierenga et al 2001, Bakshi et al 2002) and *Mycoplasma pneumoniae* (Lee et al 2002). The role of chronic infection e.g. secondary to *Chlamydia pneumoniae* (Goyal et al 2004) or tonsillitis (Ajulo 1994) remains controversial. This group of patients is also relatively immunodeficient, in part secondary to splenic autoinfarction or surgical removal of the spleen.

As well as the evidence for a possible effect of Senegal β^S-globin haplotype (Kinney et al 1999), genetic modulators of the immune (Taylor et al 2002) and host inflammatory (Hoppe et al 2001, Hoppe et al 2003) responses might also be important in the development of cerebrovascular disease and stroke. There is evidence of human leukocyte antigen susceptibility (HLA- DRB1*0301 and 0302 alleles) for stroke in SCD (Styles et al 2000). Poor diet may also affect stroke risk, probably interacting with different genetic polymorphisms. For example, increased levels of homocysteine, which may be determined by genetic and nutritional factors, were found in patients with stroke, independent of folate intake (Houston et al 1997).

Neuroimaging, cerebral angiography and transcranial Doppler ultrasound (TCD) may help to predict stroke risk. Silent infarcts on MRI are associated with a higher risk of clinical stroke (14-fold) (Pegelow et al 1995, Miller et al 2001) and progressive infarction (Miller et al 2001). Cerebral angiography demonstrating severe arterial stenosis ($\geq$ 75%) has been related to infarction, and moderate to severe arterial stenosis with deep white matter ischaemic lesions (Powars 2000, Zimmerman et al 1987). On MRA, blood flow turbulence may also be related to infarction (Seibert et al 1998). TCD studies demonstrated that in patients with SCD and MCA velocities of 200 cm/s or higher had a 40% stroke risk over three years unless they received regular blood transfusion (Adams et al 1992, Adams et al 1997, Adams et al 1998).

Hypoxia may be a risk factor for stroke by increasing haemoglobin gel formation and red cell adhesion, predisposing to molecular sickling and vaso-occlusion (Prengler et al 2002b). Acute worsening of anaemia and hypoxia is associated with a higher stroke risk (Banka et al 1977, Ohene-Frempong et al 1998, Nath et al 2000). In addition, chest syndrome is a risk factor for stroke (Ohene- Frempong et al 1998), and this respiratory complication is associated with hypoxia and may cause CNS events. Nocturnal oxyhaemoglobin desaturation is common in sickle cell disease, in young children secondary to upper airway obstruction (Walsh et al 1983, Scharf et al 1983, Sidman et al 1988, Samuels et al 1992) and appears to be associated with CNS events such as stroke, transient ischaemic attacks and seizures (Robertson et al 1988, Davies et al 1989, Kirkham et al 2001a). A study demonstrated the association of nocturnal

oxyhaemoglobin desaturation and increased numbers of platelet-erythrocyte complexes and monocytes, which enhance molecular sickling (Inwald et al 2000) and may predispose to vaso-occlusion. Endothelial attraction and adhesion of white cells, platelets, red cells and reticulocytes through upregulation of leukotriene B_4, L-selectin, P-selectin and vascular cellular adhesion molecule-1 (VCAM-1) may also be increased in chronic oxyhaemoglobin desaturation sustained during day and night (Setty et al 2003).

The mechanisms of sickling and increased red cell adhesion in association with hypoxia may be associated with red cell nidus formation and endothelial damage, leading to hyperplasia and arterial narrowing. The development of CVD might be associated with perfusion abnormalities in those patients with SCD and chronic oxyhaemoglobin desaturation.

1.10. Therapy for Stroke in Sickle Cell Disease

Transfusion therapy is the mainstay of treatment for stroke in sickle cell disease. The aim is to reduce the percentage of sickle haemoglobin and increase levels of haemoglobin A, thus reducing the deleterious effects of sickling whilst improving tissue oxygenation. For acutely sick patients, e.g. with severe chest crisis or neurological symptoms, treatment is usually simple or partial exchange–blood transfusion, avoiding rapid fluid shifts (Kirkham and DeBaun 2004). A chronic intermittent transfusion programme, aiming to keep the HbS below 30%, decreases the risk of stroke recurrence (Pavlakis et al 1989, Pegelow et al 1995, Uchida et al 1998, Prengler et al 2002b, Kirkham and DeBaun 2004). Without transfusion, the risk of stroke recurrence after the first event is up to 67% (Powars et al 1978). After stroke, blood transfusion is recommended for life (Ballas 1998, Powars 2000); however its side effects (such as antibody formation, blood borne infections and iron overload) and the practical difficulties (time in hospital, nightly Desferrioxamine injections) make it difficult for patients to tolerate this therapy in the long term. Prophylactic transfusion is recommended for patients with SCD and elevated TCD velocities (> 200 cm/sec), as the Stroke Prevention Trial in Sickle Cell Anaemia (STOP) clearly showed a reduction in strokes in this group (Adams et al 1998).

Studies of regional cerebral blood flow (CBF) using inhaled Xenon^{133} demonstrated that transfusion therapy reduced the cerebral hyperaemia in asymptomatic patients with SCD (Prohovik et al 1989), and that decreased CBF can be reversed by blood transfusion in patients with acute neurological problems (Huttenlocher et al 1984). The aim of an intensive blood transfusion regime is to keep the HbS level less than 20%-30%. Moderate transfusion regimes, maintaining HbS between 45% and 50%, were associated with a higher CBF than seen with the traditional regime, an effect apparently related in part to the haemoglobin and in part to the percentage of sickle haemoglobin (Hurlet-Jensen et al 1994). Although there were no neurological complications, the effect of the associated hyperemia over time is unknown (Hurlet-Jensen et al 1994). A study with MRA showed that blood transfusion might help to reverse CVD by increasing the lumen of the blood vessel (Steen et al 2001).

Hydroxyurea may be an alternative treatment for those who have a low tolerance for blood transfusion. The drug increases the production of foetal haemoglobin and decreases sickling (Goldberg et al 1990, Kaul et al 1995, Ware et al 1999). However, in one study, 19% of patients receiving hydroxyurea had stroke recurrence (Ware et al 1999) and in a pilot study of young children there were also recurrent CNS events (Wang et al 2001b). However, recent data from a larger series with longer follow-up suggests that hydroxyurea is an effective alternative to blood transfusion, particularly if there is overlap (Ware et al 2004). Bone marrow transplantation and allogenic umbilical cord blood stem cell transplantation may have the potential to prevent progression of CVD and stroke (Walters et al 1996, Walters et al 2000, Nietert et al 2000, Gore et al 2000, Steen et al 2001). Other therapies such as short-chain fatty acids (Kaul et al 1995), inhaled nitric oxide (Gladwin et al 1999) and arginine supplementation (Romero et al 2001) are currently under investigation. The blockade of endothelial α-V-β-3 integrin or other anti-adhesion therapies (directed to platelet integrin receptors or intravenous γ-globulin) may help to prevent vaso-occlusion (Kaul et al 2000, Harlan 2000, Hertel et al 2001). In those patients with SCD and moyamoya syndrome, revascularisation techniques (encephaloduroarteriosynangiosis or extracranial-intracranial bypass) may help to improve cerebral blood perfusion and stabilise the progression of the CVD (Vernet et al 1996, Ganesan et al 2001, Fryer et al 2004).

Finally, adentosillectomy may improve nocturnal oxyhaemoglobin desaturation in those with adenotonsillar hypertrophy and symptoms of upper airway obstruction (Davies et al 1982, Samuels et al 1992, Prengler et al 2001b), although this may not necessarily reduce the risk of neurological complications (Kirkham et al 2001a).

With respect to oxygen therapy in SCD, sickle cell adhesion may be transient (Sultana et al 1998, Wajer et al 2000) and may be reversed with hyperoxia (Nath et al 2000). One study showed that inhaled oxygen ($50\%O_2$) in sickle cell patients produced a significant reduction in the number of irreversibly sickled cells compared to those who received air. However, there was no reduction in the duration of the pain crisis in the oxygen treated patients compared to the control group (Zipursky et al 1992). Further trials are necessary to evaluate the benefits of oxygen therapy (Kirkham et al 2001a).

1.11. Hypotheses

The issues discussed in the background lead to a number of unanswered questions about the neurological complications of sickle cell disease:

a) There is uncertainty over the precise neurological diagnosis in acute events and chronic problems because the priority is to transfuse rather than to image the patient;

b) There are few data on the natural history of cerebrovascular disease (CVD) in SCD (using TCD or MRA) or abnormalities in the cerebral perfusion of these patients;

c) There has been a lack of scientific evaluation of the physiological effects of blood transfusion for patients with SCD and neurological complications e.g. improvement in the cerebral blood flow, or reversal of the vascular disease. The possibility that blood transfusion might have an aetiological role in the progression of the cerebrovascular disease has never been addressed and;

d) There is a need for further research, in order to to develop, if possible, alternative management strategies to blood transfusion, especially for the prevention of stroke and other neurological complications.

The hypotheses to be tested are the following:

- *Perfusion abnormality is associated with cerebrovascular disease and central nervous system (CNS) events in sickle cell disease.* This hypothesis is tested in a cross-sectional study (chapter 4).
- *Perfusion abnormality is associated with progression of cerebrovascular disease and central nervous system events in sickle cell disease.* This hypothesis is tested in a longitudinal study looking at whether the area of the brain in which perfusion abnormality may be demonstrated enlarges in sickle cell patients who have progression of turbulence on MR angiogram -or CVD- (chapter 5).
- *Perfusion abnormality improves with blood transfusion therapy in the short and long term.* This hypothesis is tested in a group of patients with SCD, who have had CNS events and are on blood transfusion therapy, in order to demonstrate whether the perfusion abnormality improves blood transfusion therapy by normalising the cerebral blood flow or by improving the pattern of CVD (chapter 6).
- *The genesis of seizures in sickle cell disease are associated with perfusion abnormality, vasculopathy and cerebral ischaemia.* This hypothesis is tested in a selected group of patients of this series who have had seizures during this study versus a control group (chapter 7).

Chapter 2: Subjects of this Study

2.1. Patients

Sickle cell patients with neurological symptoms were recruited from referrals of joint Haematology/ Neurology clinics attended by MP and FK in six North London hospitals (Royal London Hospital, University College Hospital, North Middlesex Hospital, Whittington Hospital, Central Middlesex Hospital, Saint Mary's Hospital). SCD patients without neurological symptoms came from a longitudinal cohort of patients, followed for more than ten years, in collaboration with Ms Alexandra Hogan, Neuropsychologist and PhD Student.

Sickle cell patients with and without neurological symptoms underwent magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), diffusion weighted imaging (DWI) and magnetic resonance (MR) perfusion (dynamic susceptibility contrast MRI [DSC-MRI]), non-imaging transcranial doppler ultrasound (TCD) and pulse oximetry at the Great Ormond Street Hospital for Children NHS Trust (GOSH) and the Institute of Child Health (ICH), UCL. Ethical permission for this research was granted by the ethics committee of GOSH and ICH. Of a total of 73 patients with sickle cell disease who underwent the neuroimaging protocol, 70 patients had a successful MR perfusion study and constituted the patient group, which was studied prospectively and retrospectively (appendix, table 1).

Of the cohort of 70 sickle cell patients, 38 were male and 32 were female; the mean age of the patients was 13.6 years (range 1 to 28 years). Sixty-seven of the 70 patients had homozygous sickle cell disease (HbSS), one had sickle cell SC disease (HbSC) and two patients had sickle cell β^0 thalassaemia disease.

2.1.1. Therapy

From the clinical data obtainable to date, 19 of the 70 sickle cell patients in this study were on a regular blood transfusion programme, 1/19 patients

on blood transfusion received overnight Oxygen, and a further two patients were also receiving antiepileptic medication. Sixteen patients had received blood transfusion previously but it had been discontinued. Of the 16 of 70 patients who stopped blood transfusion, two continued on Hydroxyurea, three received bone marrow transplantation and one patient underwent left indirect frontal revascularisation and continued on Hydroxyurea. One patient was on hydroxyurea at the start of the study. Twelve patients underwent adenotonsillectomy; one patient was also on overnight CPAP [continuous positive airway pressure]. Three patients were only on antiepileptic therapy.

2.2. Controls

2.2.1. Controls for the Cross-Sectional Study (Chapter 4)

2.2.1.1. Transcranial Doppler Ultrasound

Transcranial Doppler data were obtained from 37 controls subjects aged from 9 months to 28 years (21 males and 16 females) for the purpose of obtaining normal reference values for analysis of the TCD data. Twenty-one control subjects (11 males, 6 with sickle cell trait, 1 with alpha thalassaemia trait, and 14 without any haemoglobinopathy) came from a collaboration with the Neuropsychologist Dr Alexandra Hogan's PhD project; their age range was from 9 months to 24 years. Sixteen controls (10 males) came from a study done by Dr Fenella Kirkham and Dr Charles Newton in Kiliffi, Kenya (Newton et al 1996); their age range was from 3 years to 28 years of age and they did not have any haemoglobinopathy.

The controls from Dr Hogan's cohort included six babies who had repeated TCD studies at 9 and/or 12 months. Control data for the very young age group were necessarily sparse. Therefore, in order to increase the number of control TCD data for this age group (9-18months), the additional TCD studies of 3 babies at 9 and 12 months were included as control data, and therefore the control data for the analysis of the TCD data consisted of the number of studies and not of subjects (i.e.40 studies instead of 37 subjects).

2.2.1.2. Awake Pulse Oximetry (SpO_2)

Fifteen controls (6 with sickle cell trait, 1 with alpha thalassaemia trait, and 8 without any haemoglobinopathy) from Dr Hogan's cohort had awake pulse oximetry for the purpose of obtaining normal reference values of SpO_2. In the control group 8 subjects were male, 7 female; the mean age of the controls was 17.6 years (range 9 to 24 years). These controls subjects also had TCD and were part of the TCD control group.

2.2.2. Controls for the Study on Seizures in Sickle Cell Disease (Chapter 7)

For the study of seizures in SCD, the control group of children with homozygous sickle cell anemia had no evidence of neurological disease (n=29; 15 male) over a prolonged follow-up period but had undergone unsedated routine MRI and MRA screening (but not perfusion) over the age of six (median age 9.9, range 6.6-18.2 years) from within a clinic-based but otherwise unselected prospective cohort recruited from joint Haematology/ Neurology Clinics recruited by Dr Fenella Kirkham, Reader in Paediatric Neurology, Institute of Child Health, as a part of an ongoing study of the risk factors for stroke and cerebrovascular disease (CVD) in SCD.

2.3. Clinical Measurements: Data Collection

2.3.1. Blood Pressure

Forty-nine of 70 patients who underwent successful perfusion MRI studies had their blood pressure measured at the end of the study. In 43 patients, blood pressure was measured using a sphygmocardiograph from which systolic pressure (SP), diastolic pressure (DP) and mean arterial blood pressure (MAP) are obtained automatically; in a reduced group of patients (n= 6), only the SP and DP were measured manually with a sphygmomanometer because of technical reasons (the sphygmocardiograph was not available), in that case MAP was calculated from the formula: MAP= diastolic pressure + 1/3 pulse pressure (where pulse pressure= systolic pressure – diastolic pressure). Blood pressure was not measured in 21 patients for technical reasons or for because the

studies were not supervised directly by MP (patients from Dr Hogan's cohort or those who underwent MR studies under general anaesthesia). Control subjects did not have their blood pressure measured (patients from Dr Hogan's cohort). The normal values of blood pressure were obtained from blood pressure tables, based on normal values depending on gender and age, from a reference book on Paediatrics, *Nelson's Textbook of Pediatrics* (Nelson et al 1996).

2.3.2. Haematological Data

The haematological data consisted of haemoglobin level, haematocrit, haemoglobin S%, ferritin and iron levels, reticulocytes, white cell count and platelets, which were obtained from the clinical records of the patients from the referring hospitals. The nearest blood tests to the date of the MR studies were those used for analysis. The normal values of the haematological data were obtained from a reference book on Paediatrics, *Nelson's Textbook of Pediatrics* (Nelson et al 1996).

Chapter 3: Methodology

3.1. Magnetic Resonance Studies

3.1.1. Introduction: Perfusion MRI Using Contrast Agents (DSC-MRI)

Perfusion is the volume of blood delivered to the capillary beds of a block of tissue in a given period of time (ml/100g/min). Perfusion is the flow at the capillary level, where there is an exchange between blood and tissue. Perfusion MRI requires a tracer, either *endogenous* i.e. magnetic labelled blood (arterial spin labelling techniques) or *exogenous* i.e. a contrast agent, usually gadolinium-DTPA for a technique usually referred to as dynamic susceptibility contrast (DSC) or informally as 'bolus tracking'. After the rapid injection of gadolinium, the MRI signal loss due to spin dephasing (i.e. decrease in T2 and T2*) is measured during the fast passage of the tracer through tissue. Since the transit time of the bolus through the tissue is only a few seconds, a fast imaging technique, such as echo-planar imaging (EPI), is required to obtain sequential images during the washout and wash in of the contrast material.

The signal change (signal loss) can be related to the concentration of gadolinium, and by using a mathematical model, maps of cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) can be calculated (Figures 3.1, 3.2, 3.3). The quantification of *absolute* perfusion is not very reliable and maps of *relative* perfusion are usually used e.g. right compare with left, or grey matter compared with white matter or basal ganglia relative to cortex.

If there are delays and dispersions, the CBF, CBV and MTT maps are unreliable and it is essential to use summary parameters, such as time-to-peak (TTP), bolus arrival time (BAT), maximum peak concentration (MPC) or peak area in a semi-quantitative analysis. TTP gives information similar to MTT, MPC is approximately equivalent to CBF and peak area can be used to estimate CBV (Calamante et al 1999).

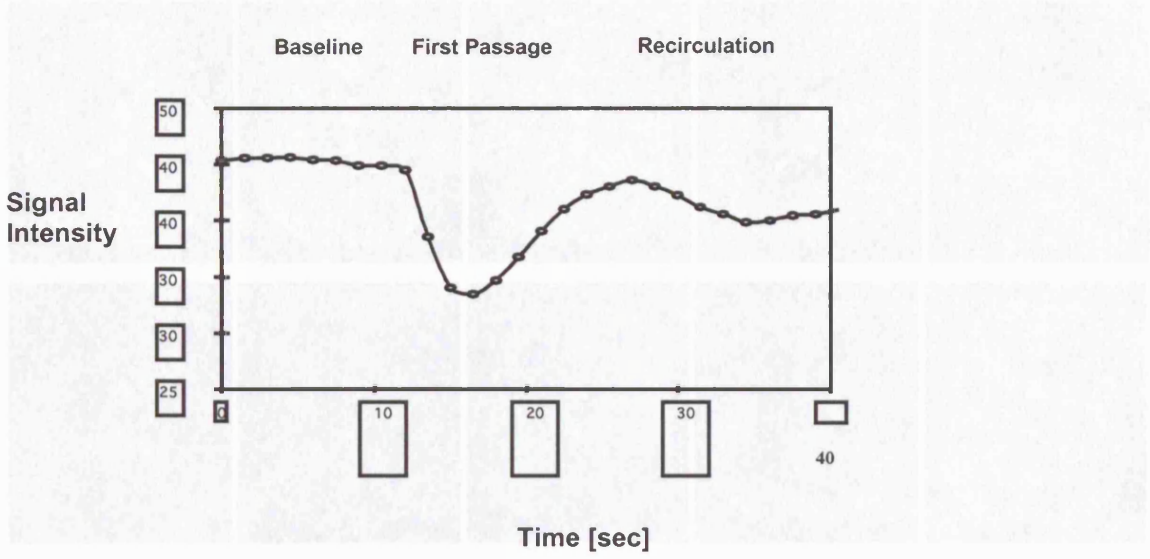


Figure 3.1. Gadolinium-signal Intensity Time Course in Perfusion MRI (DSC-MRI)

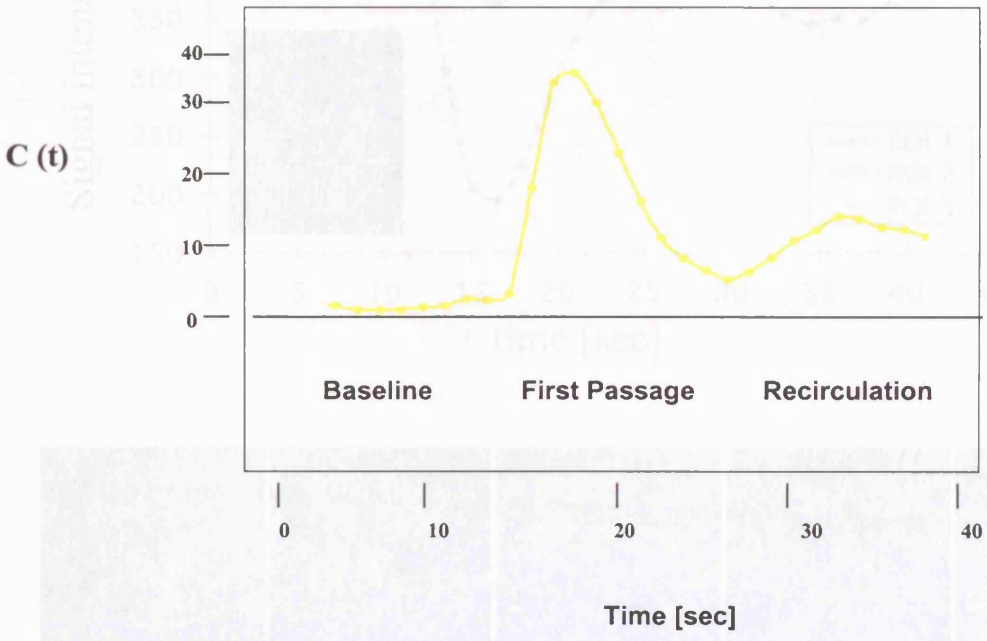
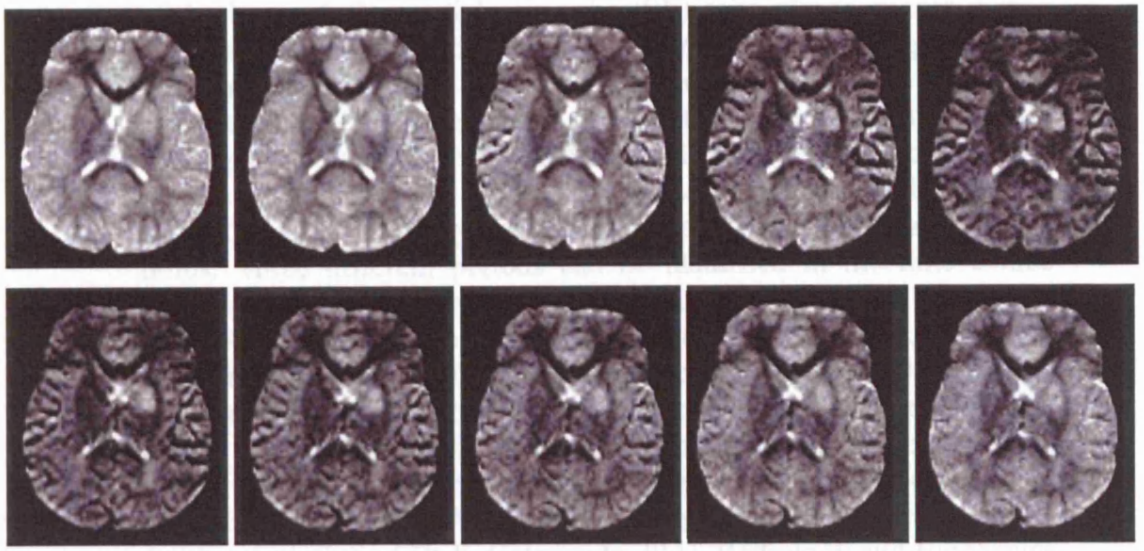
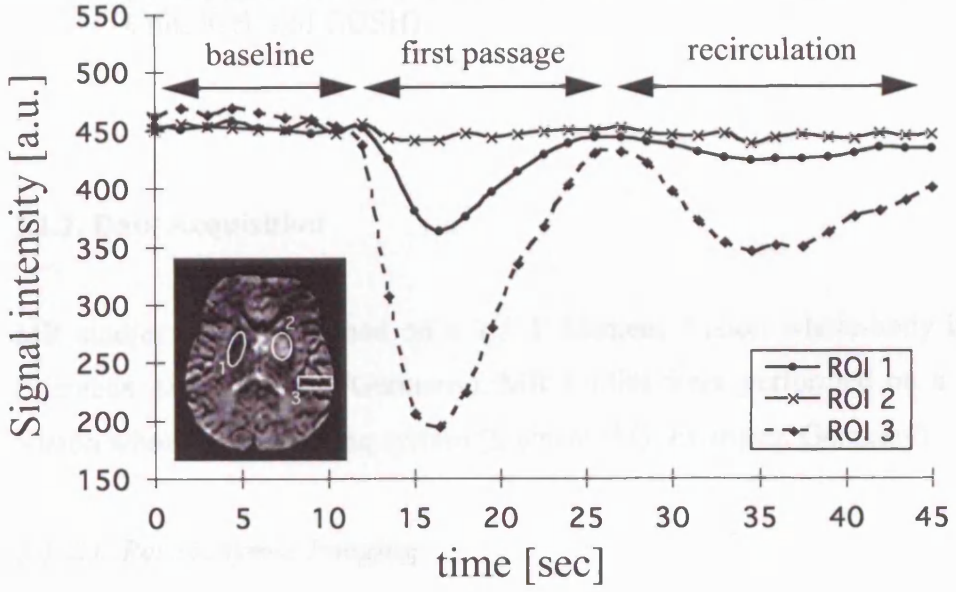


Figure 3.2. Gadolinium-Concentration Time Curve in Perfusion MRI (DSC-MRI)




baseline
first passage
recirculation
Signal intensity [a.u.]
550
500
450
400
350
300
250
200
150
0
5
10
15
20
25
30
35
40
45
time [sec]
ROI 1
ROI 2
ROI 3

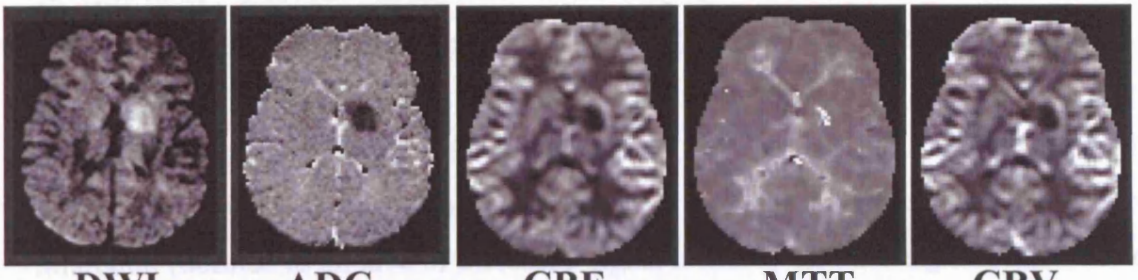

DWI
ADC$_{AV}$
CBF
MTT
CBV

Figure 3.3 (previous page). DSC-MRI in a 4 year-old child 12 h after a stroke associated with an infarct in the left basal ganglia (right side of the images). Sequential spin echo (SE) echo-planar images during the passage of a bolus of contrast agent (Gadolinium), together with the signal intensity time course (in arbitrary units) for three regions of interest (ROI [ROI 1: right basal ganglia, RO2: left basal ganglia, ROI 3: peripheral branch of the right MCA]). The top left and bottom right images correspond to t= 12 sec and 25.5 sec, respectively. The images show the signal intensity decrease associated with the passage of the bolus. Three different periods can be identified in the time course data: the baseline (before the arrival of the bolus), the first passage of the bolus, and the recircirculation period (in this case, a second smaller peak, more clearly seen in the arterial region [ROI 3]). Note that the stroke region (ROI 2) shows almost no contrast agent passage due to the very low CBF in that area. The images in the bottom row are (from left to rigtht): diffusion-weighted image, ADC_{AV}, CBF, MTT and CBV maps. All of them clearly show the ischaemic region (with permission of Dr F. Calamante, PhD, Radiology and Biophysics Unit, ICH and GOSH).

3.1.2. Data Acquisition

MR studies were performed on a 1.5 T Siemens Vision whole-body imaging system (Siemens AG, Erlangen, Germany). MR studies were performed on a 1.5 T Siemens Vision whole-body imaging system (Siemens AG, Erlangen, Germany).

3.1.2.1. Parenchymal Imaging

Seventy-three patients underwent neuroimaging studies. The structural MRI investigation included sagittal and coronal T1-weighted images (TR=570 msec, TE= 14 msec), axial TSE T2-weighted (TR=3,458 msec, TE= 96 msec), and coronal Turbo-FLAIR T2- weighted images (TR= 9,999 msec, TE= 119 mesc, TI = 2,210 msec). MRI abnormalities were classified as overt cerebral infarction (stroke), silent or covert infarction (defined as an area of increased signal intensity on T2-weighted MRI without a history of a neurological event lasting more than 24 hours [Moran et al 1998]), and cerebral atrophy. MRI studies were assessed by two Neuroradiologists (DS and KC).

3.1.2.2. Magnetic Resonance Angiography

MR Angiogram (MRA) was performed using a three-dimensional (3D) time-of-flight method, acquiring 3 slabs, each of 3.2 mm thickness, centred on the circle of Willis (TR= 35 msec, TE= 7.2 msec, flip angle = 20°).

The MR angiograms were reviewed by at least two of four neuroradiologists (WKC, DS, TC, MB), who examined them carefully following published criteria (Kirkham et al 2001b) for evidence of turbulence in the terminal internal carotid (TICA), A1 (anterior cerebral artery), A2, M1 (middle cerebral artery), M2, P1 (posterior cerebral artery) and P2 segments of the basal vessels (figures 3.4, 3.5, and 3.6). Turbulence on the MRA was graded as 1 (mild), 2 (moderate), 3 (severe), 4 (occlusion) and 5 (occlusion plus collaterals [moyamoya syndrome] figure 3.7). The presence of collaterals was also documented (Kirkham et al 2001b).

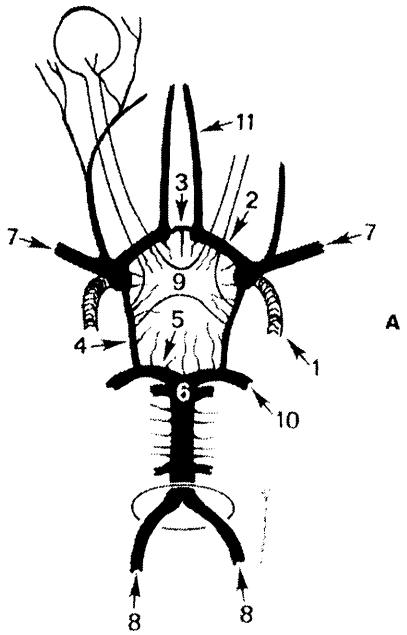


Figure 3.4 Anatomic diagram of the circle of Willis. 1: internal carotid artery (ICA); 2: horizontal (A1) anterior cerebral artery (ACA) segment; 3: anterior communicating artery; 4: posterior communicating artery; 5: P1 segment of posterior cerebral artery (PCA); 6; basilar artery bifurcation; 7: middle cerebral artery (MCA; not part of the circle of Willis); 8: vertebral arteries (not part of circle of Willis); 9: optic chiasm; 10: P2 (post-communicating) PCA

segment; 11: A2 (post-communicating) ACA segment (modified from Davies and Jacobs 1994).

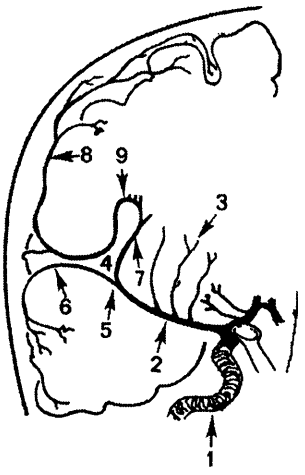


Figure 3.5. Anteroposterior anatomic drawing of the middle cerebral artery (MCA) and its branches. 1: internal carotid artery; 2: horizontal (M1) MCA segment; 3: lateral lenticulostriate arteries; 4: Sylvian fissure; 5: MCA bifurcation; 6: anterior temporal artery; 7: M2 (sylvian) segments of MCA hemispheric branches; 8: M3 (opercular) MCA branches; 9: Sylvian point (from Davies and Jacobs 1994).

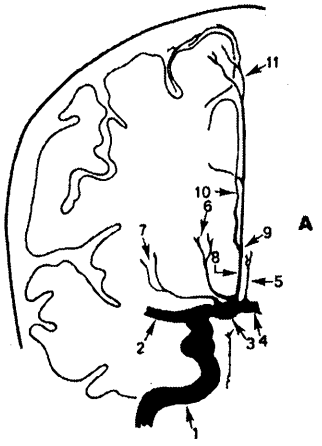


Figure 3.6. Anteroposterior anatomic drawing of the anterior cerebral artery (ACA) and its branches. 1: Internal carotid artery; 2: middle cerebral artery; 3: horizontal (A1) ACA segment; 4: anterior communicating artery (ACoA); 5: small ACoA branch to basal ganglia, corpus callosum genu; 6: medial lenticulostriate arteries; 7: recurrent artery of Heubner; 8: A2

segment of ACA; 9: ACA bifurcation; 10: pericasollal artery; 11: callosomarginal artery (from Davies and Jacobs 1994).

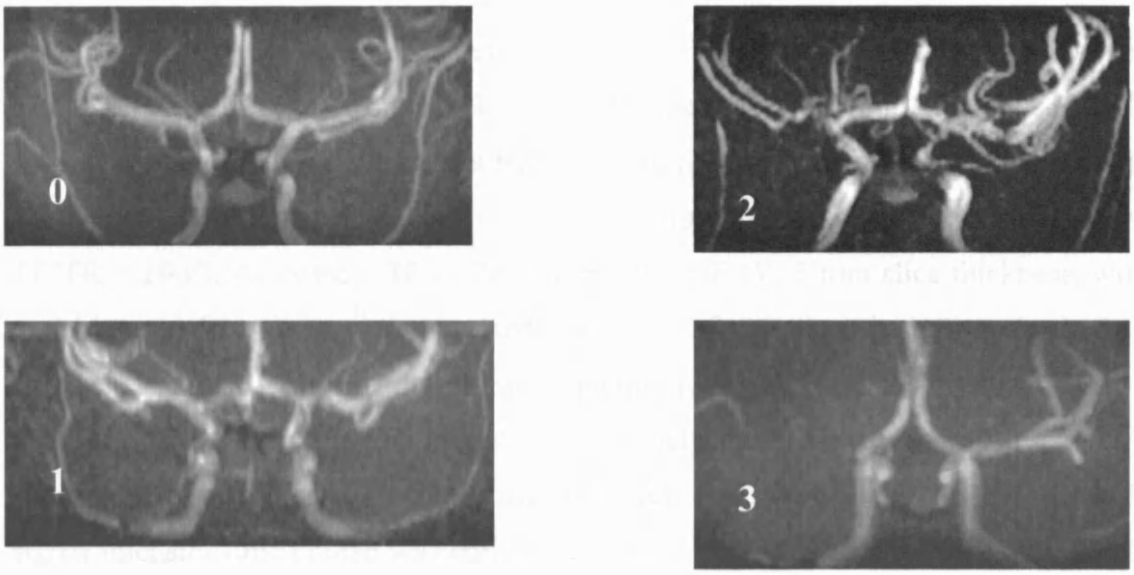


Figure 3.7. Magnetic resonance angiograms (MRAs) from four patients with sickle cell disease showing different grades of MRA turbulence. 0: normal; 1: mild; 2: moderate; 3: severe.

3.1.2.3. Diffusion Weighted Imaging

Diffusion weighted imaging (DWI) was performed using a FLAIR spin-echo EPI sequence, with a pair of diffusion gradients on either side of the refocusing pulse. The imaging parameters were TE/TR = 86/8,7000 msec, inversion time (TI) = 2,100 msec, 128 x 128 matrix, 24 cm FOV, 5 mm slice thickness, 1 mm gap between slices. The diffusion parameters were: diffusion gradient duration (δ) = 15 msec, time interval between diffusion gradients (Δ) = 40.2 msec, b values = 0 and 167 sec/mm^2. A multislice reference scan was acquired for the on-line correction of B_0 eddy-current effects (Calamante et al 1999). Maps of the apparent diffusion coefficient (ADC) were calculated online in three orthogonal directions, which were combined to generate

average ADC maps (ADC_{AV}) to eliminate the confounding effects of diffusion anisotropy (Ulug et al 1997).

3.1.2.4. Perfusion MRI (DSC-MRI)

Seventy of 73 patients had successful perfusion MRI. Dynamic susceptibility contrast MRI (DSC-MRI) or perfusion MRI was performed using a multislice spin-echo EPI sequence to follow the passage of a bolus of gadolinium diethylenetriaminepenta-acetic acid (Gd-DTPA; Magnevist, Schering AG, Germany). The imaging parameters were TE/TR = 100/1,500 msec, 128 x 128 matrix, 24 cm FOV, 5 mm slice thickness, with a variable gap. Six slices were acquired, with one of the slices including the MCA to allow the estimation of the arterial input function (AIF). A Gd-DTPA bolus of 0.1-0.15 mmol/kg body weight was injected intravenously (rate 2-6 ml/sec) using an MR-compatible power injector (Medrad Inc, Pittsburgh, PA), followed by a saline flush. The signal intensity time course was converted to a concentration time course, and the AIF was used to deconvolve the concentration time curve using singular value decomposition (SVD). A 3 x 3 uniform smoothing kernel was applied to the raw image data before deconvolution. Maps of cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) were calculated on a pixel-by-pixel basis. When long bolus arrival delays are present in the areas with perfusion disturbance, the quantification of DSC-MRI data using SVD produces very inaccurate results. In these cases, time-to-peak (TTP) maps were calculated on a pixel-by-pixel basis. A region was considered to be abnormal on DSC-MRI when at least one of the following situations was observed on visual inspection: focal reduction in CBF (mild [denoted as a minus symbol: -], moderate [- -] or severe [- - -]), increase in MTT (mild [denoted as a plus symbol: +], moderate [+ +] or severe [+ + +]), reduction or increase in CBV (mild [-] moderate [- -] or severe [- - -]), or increase in TTP (mild [+], moderate [+ +] or severe [+ + +]; figures 3.8, 3.9 and 3.10 [Calamante et al 2001, Kirkham et al 2001b]).

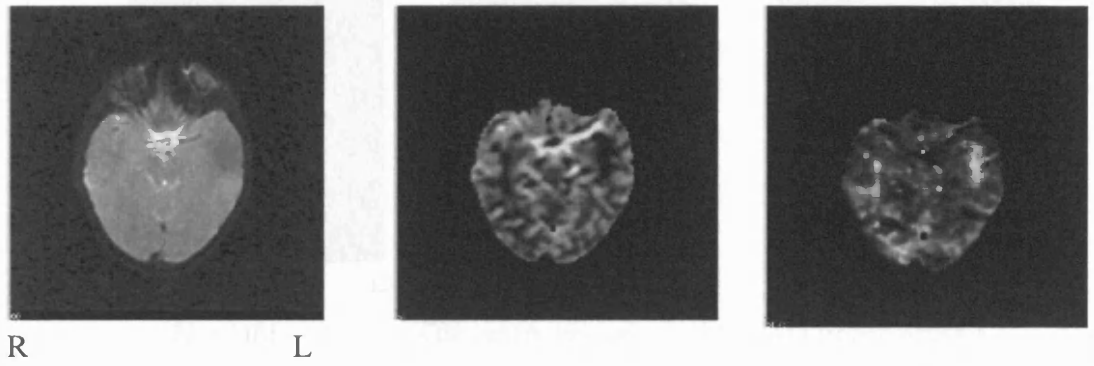


Figure 3.8. Perfusion MRI (DSC-MRI) in a patient with sickle cell anaemia and seizures. Normal T2-weighetd MRI; mild decrease in cerebral blood flow (CBF) or (-), mild increase in the mean transit time (MTT) or (+) in the right temporal areas. Cerebral blood volume (CBV) maps are similar to CBF.R: right; L: left.

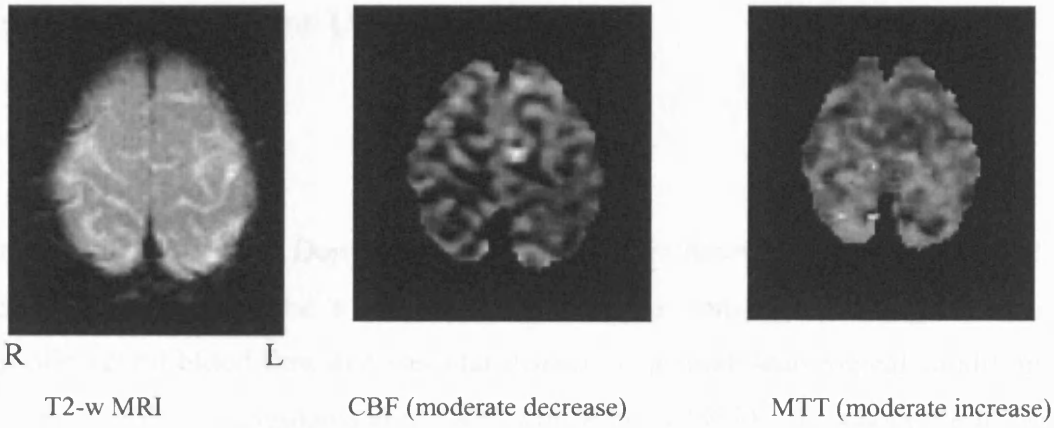


Figure 3.9. Perfusion MRI (DSC-MRI) in a patient with sickle cell anaemia, posterior territory TIA (double vision) and headaches. TCD MCA velocities were > 200 cm/sec. Normal T2-weighted MRI; moderate decrease in cerebral blood flow (CBF) or (- -), moderate increase in the mean transit time (MTT) or (+ +) in frontal parietal areas bilaterally. R: right; L: left. Cerebral blood volume (CBV) maps are similar to CBF.

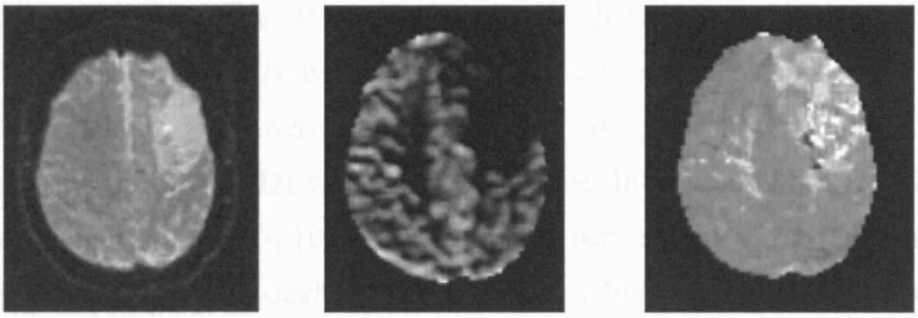


Figure 3.10. Perfusion MRI (DSC-MRI) in a patient with sickle cell anaemia and stroke. Left frontal infarct on T2-weighted MRI; severe decrease in cerebral blood flow (CBF) or (- - -), severe increase in the mean transit time (MTT) or (+ + +) in the left frontal areas. Cerebral blood volume (CBV) maps are similar to CBF. R; right; L: left.

3.2. Transcranial Doppler Ultrasonography

3.2.1. Introduction

The technique of transcranial Doppler ultrasound was first described by Aaslid (1982) and since then, it has become a very useful tool for a non-invasive diagnosis and screening of cerebral blood flow and vascular disease in several neurological conditions either acutely or follow-up (Adams et al 1992, von Reuten 1993). It is possible to use transcranial Doppler ultrasound (TCD) to measure mean arterial blood flow velocities in the distal internal carotid/proximal middle cerebral artery, the main site of the large-blood vessel disease, because of the small angle between vessel and probe which means that velocity is directly related to the Doppler shift frequency (Aaslid et al 1982, Padayachee et al 1986, Kirkham et al 1986, Gillard et al 1986, figure 3.11).

Increased TCD velocities may be due to a reduction in artery diameter (Adams et al 1992), or increased CBF in the presence of anaemia (Brass et al 1988, Adams et al 1989). In African children with normal haemoglobin, the upper limit (+2 standard deviations from the mean) for MCA velocity was 142 cm/sec even when there was iron deficiency anaemia (Newton et al 1996), and the lower limit (-2 standard deviations from the mean) was 42 cm/sec (Newton et al 1996) in a study which included young infants in whom velocities are lower (Bode et al 1988). For children in this population aged 7-14 years, the mean (+/-SD) was 90 +/-20 cm/sec. In children with HbSS, the MCA velocity is typically between 100-130 centimeters per second (cm/sec) (Adams et al 1989). Although children with sickle cell disease have a 40 to 50 percent higher mean velocity of flow than children without anaemia, angiographic correlation has shown that severe stenosis is associated with TCD velocities 2 to 3 times normal (Adams et al 1992). In sickle cell anaemia, stenosis on conventional arteriography may be detected when the MCA velocities are between 140 and 190 cm/sec; velocities more than 190 cm/sec are associated with marked artery stenosis on angiography (Adams et al 1992).

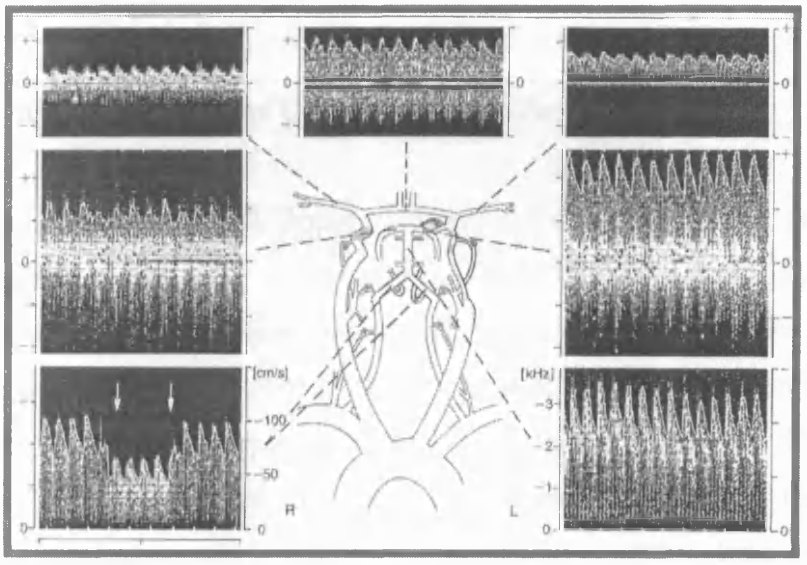


Figure 3.11. Transcranial Doppler ultrasound (TCD) measures mainly mean arterial blood flow velocities in the distal internal carotid/proximal middle cerebral artery (the main site of the large-blood vessel disease). From von Reuter and von Budingen, 1993.

Mean MCA blood flow velocities equal or greater than 200 cm/sec are associated with a 40 % of risk of stroke over the subsequent three year-period (13% per year) if patients

do not receive blood transfusion therapy (Adams et al 1992, 1997 and 1998). TCD velocities equal to or more than 170 cm/sec are considered conditional, predicting a stroke risk of 7% over the subsequent 3 years; on the other hand, patients with TCD velocities between 80 and 170 cm/sec had a 2 % risk of stroke over the same period (Adams et al 1997). In another series of sickle cell patients, TCD velocities > 200 cm/sec or less than 100 cm/sec or presence of arterial occlusion (no flow) were found in 9 out 12 patients with cerebral infarct (Siegel et al 1995).

The anterior cerebral artery (ACA) velocity is usually ≤80% of the velocity of the MCA on TCD but velocity can exceed 120% of the MCA velocity in the presence of intracranial or extracranial pathology (Adams et al 1992b). Following Adams's criteria, ACA or MCA stenosis is likely when there is a high ipsilateral ACA/MCA ratio equal or more than 1.2; the increased ACA velocity may be either associated to turbulent flow secondary to the same artery stenosis or secondary to increased collateral flow because of MCA/ ICA occlusion (Adams et al 1992b).

3.2.2. Transcranial Doppler Ultrasound Data Acquisition

A non-imaging transcranial Doppler ultrasound (TCD) (2 MHz probe, Nicolet EME, Germany) was used by MP to insonate the distal internal carotid (ICA), middle (MCA), anterior (ACA) and posterior cerebral (PCA) and basilar arteries using a previously described protocol (Adams et al 1992, Adams et al 1997, Kirkham et al 2001) in 68 out of 70 patients with successful perfusion MRI and 21 control subjects. The MCA, distal ICA, ACA and PCA were studied with the transtemporal approach, and the basilar artery with the suboccipital approach. Time-averaged mean cerebral blood flow velocities were recorded in centimeters per second (cm/sec). Mean MCA velocity was recorded every 2 mm at depths of 40 to 60 mm (Adams et al 1992). The maximum time averaged velocity towards the probe (ICA/MCA) was documented on both sides following Adams' criteria (Adams et al 1997). For analysis, the higher velocity of the two sides was taken (Kirkham et al 2001).

TCD findings were abnormal if one or more of the following findings were present: 1) mean MCA velocity less than 70 cm/sec and MCA ratio (lowest: highest) velocity < 0.5;

or an ACA: MCA mean velocity ratio greater than 1.2; 2) mean MCA velocity greater than 170 cm/sec and less than 200 cm/sec, deemed conditional by Adams and colleagues (1997); 3) a mean MCA velocity equal or greater than 200 cm/sec deemed critical and premonitory for stroke by Adams and colleagues (1997); 4) undetectable MCA (Bode et al 1988, Adams et al 1992b, Newton et al 1996, Adams et al 1997, Kirkham et al 2001, Zafeiriou et al. 2004, figures 3.12.1, 3.12.2, 3.12.3, 3.12.4, 3.12.5).

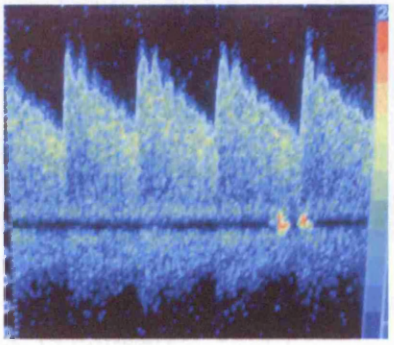


Figure 3.12.1. Normal transcranial Doppler ultrasound (TCD): normal mean middle cerebral MCA velocities (figure: MCA 83 cm/sec)

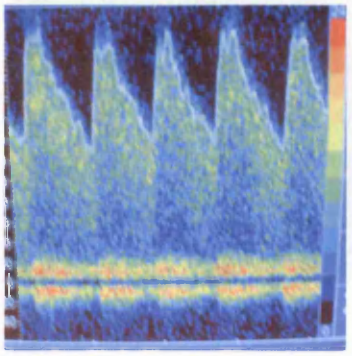


Figure 3.12.2. Conditional TCD: mean MCA velocities $\geq 170 < 200$ cm/sec (figure: MCA 182 cm/sec).

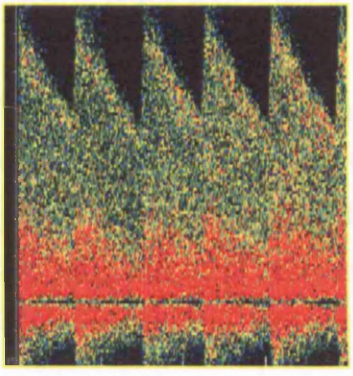


Figure 3.12.3. Critical MCA velocity on TCD: mean MCA velocities > 200 cm/sec. (figure: MCA 220 cm/sec).

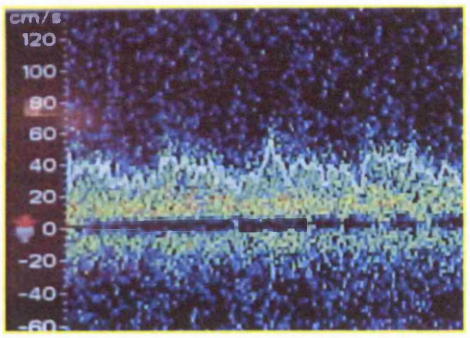


Figure 3.12.4. Low velocities on TCD: mean MCA velocities < 70 cm/sec. (figure: MCA 35 cm/sec)

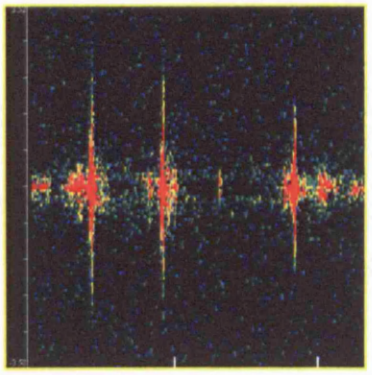


Figure 3.12.5. Undetectable signal on TCD: Undetectable MCA (signal by artifact or very low blood flow: MCA 8-10 cm/sec).

3.3. Awake Oxygen Saturation (SpO_2): Measurement Methodology

3.3.1. Awake Pulse Oxymetry Methodology and Data Acquisition

Daytime, awake pulse oximetry – SpO_2 and pulse rate (Minolta PulsOx 3i, Stowood Scientific Instruments, Oxford, England) was recorded for three minutes in 57 of 70 patients with successful perfusion MRI and 14 of 15 controls. A baseline SpO_2 of less

than 92% was set as a cut-off for oxyhaemoglobin desaturation (Beckerman et al 1992, Ferber and Kryger 1995).

3.4. Neurophysiology

An electroencephalogram (EEG) was recorded in patients who had seizures, using standard digital EEG equipment (Nicolet 'Bravo'), and electrode placements. EEGs were reported using conventional clinical methods of visual inspection (in chapter 7).

3.5. Statistical Analysis

The statistical analysis of the data varied for each study and is therefore described in each chapter.

Chapter 4: Association of Perfusion Abnormality with Cerebrovascular Disease and Central Nervous System Events in Sickle Cell Disease: A Cross-Sectional Study

4.1. Introduction and Aims

4.1.1. Introduction

Cerebral perfusion has been previously studied in sickle cell disease in small groups of patients, with different techniques in cross-sectional studies. Previous studies assessed either cerebral blood flow haemodynamics in SCD or compared areas of cerebral blood flow (CBF) abnormality with structural imaging (MRI) in symptomatic versus asymptomatic patients (or control subjects) using different techniques such as inhaled $Xenon^{133}$ (Banka et al 1977, Huttenlocher et al 1984, Prohovnik et al 1989, Kluger et al 1993), positron emission tomography -PET- (Herold et al 1986, Rodgers et al 1988, Powars et al 1999) and T2*-weighted MRI (Tzika et al 1999). In addition, a few investigators studied the effect of blood transfusion on cerebral perfusion using inhaled $Xenon^{133}$ techniques (Prohovnik et al 1989, Venketasubramanian et al 1994, Hurlet-Jensen et al 1994; see chapter 6).

With the development of new MR techniques which allow the assessment of cerebral perfusion without exposing the patient to radioactivity, two recent cross-sectional studies demonstrated the sensitivity of perfusion MRI in patients with sickle cell disease. One study reported cerebral perfusion abnormalities in symptomatic patients with SCD using dynamic susceptibility contrast MRI (DSC-MRI) and compared these findings with other modalities such as MRI, DWI, MRA and TCD (Kirkham et al 2001). The study found regions of abnormal perfusion which were normal on structural neuroimaging; moreover, in some patients, the extent of the perfusion abnormality was beyond the areas of the ischaemic lesions found on MRI (Kirkham et al 2001). The other study using continuous arterial spin- labelling perfusion MRI, which measures cerebral blood flow (Oguz et al 2003), demonstrated that CBF was higher in sickle cell patients in relation to controls and that there were cerebral perfusion abnormalities in

sickle cell patients who had normal MRI, although some of these patients had cognitive symptoms.

However, although Kirkham et al reported a preliminary study comparing different imaging techniques and perfusion MRI (Kirkham et al 2001), the patterns of perfusion abnormality in relation to the neurological symptoms in patients with SCD have not been described in parallel with the severity of the perfusion abnormality compared with the grade of abnormality seen on conventional neuroimaging or on TCD. In addition, the effect of blood pressure and haematological parameters on cerebral perfusion in SCD has not been well documented, although it would be very important to determine whether these parameters are associated with perfusion abnormality in this population.

4.1.2. Aims of the Study

The aim of this cross-sectional study was to document the cerebral perfusion abnormality and compare it with the severity of cerebrovascular disease and with a variety of clinical central nervous system events in sickle cell disease.

The hypothesis to be tested in this cross-sectional study was that, in patients with sickle cell disease (SCD), there were different degrees of severity of the cerebral perfusion abnormality, and these were related to different neurological symptoms and the severity of the cerebrovascular disease.

The clinical questions arising from this hypothesis involved examining whether in sickle cell disease there were associations between:

1. The clinical severity of the central nervous system events and the severity of the cerebral perfusion abnormality in SCD
2. The clinical severity of the central nervous system events and conventional neuroimaging (T2-weighted MRI and MRA turbulence)
3. The severity of the cerebral perfusion abnormality and the findings on MRI and grade of turbulence in the intracranial vessels on MRA

4. Intracranial vessel cerebral blood flow velocity measured using transcranial Doppler ultrasound (TCD) and turbulence documented using MRA in the same vessels.
5. Abnormal TCD and perfusion abnormality, to examine whether TCD could be used as a non-invasive method of monitoring cerebral blood flow in SCD
6. Blood pressure and neurological symptoms in SCD
7. Blood pressure and cerebral infarction,
8. Blood pressure and cerebrovascular disease
9. Blood pressure and cerebral perfusion
10. Haematological parameters, such as haemoglobin, white cell count, platelets and haemoglobin S%, and cerebrovascular disease in SCD
11. Haematological parameters and cerebral infarction
12. Haematological parameters and cerebral perfusion abnormality

In addition to, and in part as a result of addressing all of the above questions, the over-riding aim was to determine if it is possible to identify predictors of recurrent neurological symptoms from cross-sectional MRI, MRA, TCD and MRI perfusion studies in patients with SCD who have already experienced central nervous system complications.

4.2. Subjects

4.2.1. Patients

Data collection in the patients of this study has been described in chapter 2 (Appendix Table 1). Sickle cell patients with neurological symptoms were recruited from referrals of joint Haematology/ Neurology clinics attended by MP and FK in six North London hospitals. SCD patients without neurological symptoms came from a longitudinal study of a cohort of patients, followed for more than ten years, in collaboration with Alexandra Hogan, Neuropsychologist and PhD Student. Ethical permission was granted by the committee of Great Ormond Street Hospital for Children NHS Trust.

Sickle cell patients with and without neurological symptoms underwent MRI, MRA, DWI and MRI perfusion, non-imaging TCD and pulse oximetry at Great Ormond Street Hospital for Children NHS Trust (GOSH) and the Institute of Child Health (ICH), UCL. Seventy (67 with neurological symptoms) of 73 patients had a successful MR perfusion study and constituted the patient group that was studied. Sixty-seven out of 70 patients had homozygous sickle cell disease (HbSS), one had sickle cell SC disease (HbSC) and two patients had sickle cell β^0 thalassaemia disease. The series of 70 patients with sickle cell disease with their clinical, neuroimaging and TCD data are shown in Appendix Table 2; the patients are numbered following a decreasing order of severity of their neurological symptoms.

Of the cohort of 70 sickle cell patients, 38 were male and 32 were female; the mean age of the patients was 13.6 years (range 1 to 28 years).

4.2.2. Controls

4.2.2.1. Trancranial Doppler Ultrasound

Transcranial Doppler data were obtained from 37 controls subjects aged from 9 months of age to 28 years (21 males and 16 females) for the purpose of obtaining normal reference values for comparison with the TCD data from the patients with sickle cell disease. Twenty-one control subjects (11 males; 6 with sickle cell trait, 1 with alpha thalassaemia trait, and 14 without any haemoglobinopathy) came from the collaboration with the Neuropsychologist Dr Alexandra Hogan; their age range was from 9 months to 24 years. Sixteen controls (10 males) came from a study done by Dr Fenella Kirkham and Dr Charles Newton in Kiliffi, Kenya (Newton et al 1996); their age range was from 3 years to 28 years of age and they did not have any haemoglobinopathy.

The controls from Dr Hogan's cohort included six babies who had repeated TCD studies at 9 and/or 12 months. Control data for the very young age group were necessarily sparse. Therefore, in order to increase the number of control TCD data for this age group (9-18months), the additional TCD studies of 3 babies at 9 and 12 months were included as control data, and therefore the control data for the analysis of the TCD

data consisted of the number of studies and not of subjects (i.e. 40 studies instead of 37 subjects).

4.2.2.2. Awake Pulse Oximetry (SpO_2)

Fifteen controls (6 with sickle cell trait, 1 with alpha thalassaemia trait, and 8 without any haemoglobinopathy) from Dr Hogan's cohort had awake pulse oximetry for the purpose of obtaining normal reference values of SpO_2. In the control group 8 subjects were male, 7 female; mean age of the controls was 17.6 years (range 9 to 24 years). These controls subjects also had TCD and were part of the TCD control group.

4.3. Methods

4.3.1. Conventional Neuroimaging, Perfusion MRI, Transcranial Doppler Ultrasound, Oxygen Saturation, Blood Pressure Measurements and Haematological Parameters

The patients underwent MRI, MRA, DWI, and perfusion MRI studies following a published protocol (Kirkham et al 2001) with simultaneous 3-minute pulse oximetry, transcranial Doppler ultrasound and measurement of the blood pressure performed after the perfusion MRI. The data acquisition for these studies is described in chapter 3. In addition, the available haematological data and blood pressure measurements (of those patients who did not have the blood pressure recorded at the time of their MR studies) were collected from the clinical records of the patients.

As was mentioned in chapter 3, by convention at the Radiology and Physics Unit at ICH/GOSH, abnormal perfusion MRI was characterised by a regional increased MTT which was defined with a sign '+' on visual inspection (=: normal ; +: mild ; + +: moderate; + + +: severe increase in MTT); a regional decreased CBF and/ or CBV was defined with a sign '-' (=: normal; -: mild decrease; - - : moderate decrease; - - - : severe decrease in CBF or CBV); or a regional increase in CBF and/ or CBV (=: normal; +: mild; + +: moderate; + + + : severe increase in CBF or CBV, Calamante et al 1999). An

increase of MTT and/or decreased CBF or CBV is seen when there is cerebrovascular disease with artery stenosis or occlusion (Kirkham et al 2001) leading to slowing of the passage of blood flow secondary to the arterial abnormality. Two of the 70 patients (3%) (both female, 16 and 22 years old) had side effects secondary to the intravenous bolus of Gadolinium, which consisted of vomiting in one patient and nasal congestion and cough with mild bronchospasm in the other. The symptoms were transient and the patients rapidly improved, in the first case spontaneously, and in the second with nasal Oxygen.

4.3.2. Data Analysis

Statistical analysis was undertaken using SPSS 10 for Windows. For the study of association among recurrent neurological symptoms, conventional neuroimaging (MRI and MRA), TCD and perfusion MRI, χ^2 and Fisher's exact test were used for binary categorical data, the Mann-Whitney test for associations between binary data and ordinal data, and Spearman's test for ordinal data. For clinical data (blood pressure, haematological parameters and oxygen saturations) logistic regression was used for association between continuous data and binary categorical data in relation to an outcome. One-way analysis of variance [ANOVA] was used to compare means of continuous data. Tukey's test (ANOVA) was used for post-hoc analysis between mean values of continuous data to analyse differences in mean values by age group or by clinical symptoms. Wilcoxon's test for related samples was used to compare non-parametric continuous data for analysis of TCD velocities within groups. Statistical significance was defined as $p<0.05$, and a trend for significance was defined as $p \geq 0.05$ and $p \leq 0.1$.

4.4. Results I: Neurological Symptoms and Clinical Parameters

4.4.1. Neurological Symptoms

4.4.1.1. Central Nervous System Events at Presentation

The clinical symptoms of the patients of this study are described in those who had a successful perfusion MRI study (n= 70 patients). The main neurological syndrome at presentation was described in every patient with the clinical data obtained to date and

other recurrent symptoms (such as headaches, seizures and learning difficulties) considered as secondary features if they were not the main neurological symptom for a given patient (figure 4.1).

As the main neurological symptom at presentation, 4 patients (6%) had coma (2 with underlying stroke accompanied by coma with cerebral oedema and 2 associated with symptoms of posterior territory transient ischaemic attack). Fourteen patients had stroke (overt infarction, 20% [including the 2 patients who had coma and stroke, the overall stroke prevalence was 23%]), mostly presenting with focal neurological symptoms such as hemiparesis or visual symptoms.

Twenty-three of 70 patients (33%) had transient ischaemic attacks (TIA), 9 with anterior territory TIA characterized mainly by transient hemiparesis and/or hemiparaesthesia. The other 14 patients had posterior territory TIA; symptoms were characterized by headaches, visual symptoms, dizziness and confusion; in a lesser degree accompanying hemiparesis in one patient and seizures in another.

Seven of 70 patients (10%) presented with seizures at onset, which included blank spells (n=3), staring spells (n=1), generalised tonic-clinic seizures (n=2), and visual phenomena (n=1).

Fifteen patients (21.4%) had headaches, and 4 patients (6%) had learning difficulties as main symptoms at presentation. Only 3 patients (4%) did not have neurological symptoms but had severe SCD with recurrent crises such as chest syndrome.

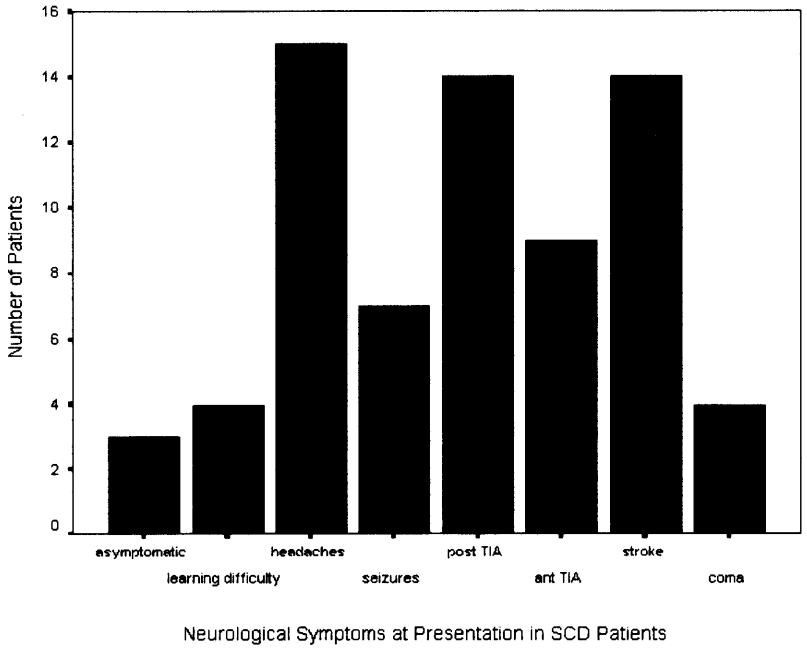


Figure 4.1. Frequency of different central nervous system events at presentation of sickle cell patients who had successful perfusion MRI

4.4.1.2. Recurrent Neurological Symptoms

The main recurrent symptom (most severe recurrent symptom) in these patients with sickle cell disease included (figure 4.2): stroke in 3 patients (4%), one of whom had accompanying symptoms of posterior leukoencephalopathy with coma and hypertension; all were on chronic blood transfusion, although one of these patients stopped blood transfusion due to antibody formation against blood red cells. Other recurrent symptoms included: reversible ischaemic neurological deficit –RIND- (defined as the persistence of focal neurological symptoms lasting more than 24 hours but eventually recovering completely) in one patient (1%); and TIAs in 11 (16% [7 anterior and 4 posterior]). In addition, 7 patients (10%) had recurrent seizures, 31 patients (44%) had headaches, and a further 6 (9%) had learning difficulties. Only eleven patients (16%) did not have any documented recurrent neurological symptoms.

Of the sickle cell patients who had recurrent symptoms, some had only one recurrent symptom, while other had multiple recurrent symptoms. Other recurrent neurological symptoms accompanying the main recurrent symptom included TIAs (n=2); seizures (n=1); headaches (n=15); and learning difficulties /behaviour problems in 32 patients.

Twenty-three of 70 patients (33%) had only a main recurrent neurological symptom. Furthermore, 36 patients (51%) had two or more recurrent symptoms: 24 (34% of 70 patients) with two recurrent neurological symptoms, 10 (14%) with three recurrent symptoms, and 2 patients (3%) with four different recurrent symptoms.

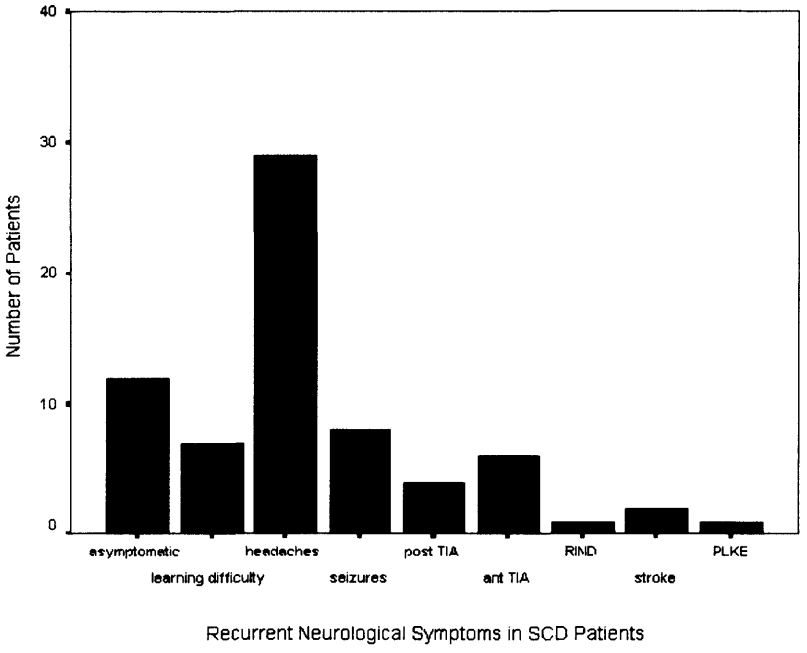


Figure 4.2. Frequency of the main recurrent neurological symptoms of the sickle cell patients who had a successful perfusion MRI.

4.4.1.3. Inter-Observer Assessment in the Short- and Long- Term for Neurological Symptoms

Two paediatric neurologists who attended the joint Haematology/Neurologist clinics assessed the sickle cell patients of this series. Observer 1 was the author and observer 2 was a Consultant Paediatric Neurologist (FJK). Observer 1 assessed the sickle cell patients over a relatively short period of time (3 years), as her observations on the patients' neurological symptoms were based on notes taken during the clinics and/or during her supervision of the patients' MRI perfusion studies, and/or from the referral letters written by the Consultant Paediatricians or Haematologists. Observer 2 was the Consultant Paediatric Neurologist for these patients who had followed and assessed them over a longer period of time (up to 14 years). Both observers completed forms that

had a classification of neurological symptoms, highlighting the most severe neurological symptom at presentation and recurrence for each patient, which were used for the analysis of the clinical data. Whenever accurate clinical data were not available for a patient, the form was left blank (10 patients for observer 1).

Figures 4.3 and 4.4 show the inter-observer assessment in the short- and long-term for neurological symptoms at onset, which revealed that there was a high correspondence (82%) for stroke, whereas for the other symptoms of lesser severity, the correspondence was around 50%. Similarly, the inter-observer assessment in the short- and long-term for recurrent neurological symptoms (figures 4.5 and 4.6) had a very high correspondence for the most severe symptoms such as coma, stroke and RIND (100%). However for the other symptoms, the agreement was around 50%, the highest agreement was for headaches (62%) and the lowest agreement was for learning difficulty (36%).

It is possible to argue that long-term follow-up might be more accurate for the description of neurological symptoms for these patients, especially for detection of symptoms of lesser severity. The restriction of time during a single consultation or during the supervision of the patient during the MR studies may jeopardize the quality of clinical history taking, as could the fact that these symptoms were not always described in the referral letters. In addition, sickle cell patients may change their neurological symptoms over time (with worsening or improvement in symptomatology) depending on the course of their disease, and may forget previously experienced symptoms which have resolved completely.

Figure 4.3. Inter-observer assessment in the short- (observer 1) and long-term (observer 2) of the neurological symptoms at presentation of each sickle cell patient – cross- sectional data. Categories of symptoms: 0= none; 1= learning difficulty (cognitive symptom); 2= headaches; 3= seizures; 4= posterior territory transient ischaemic attack (TIA); 5= anterior territory TIA; 6= stroke; and 7= coma.

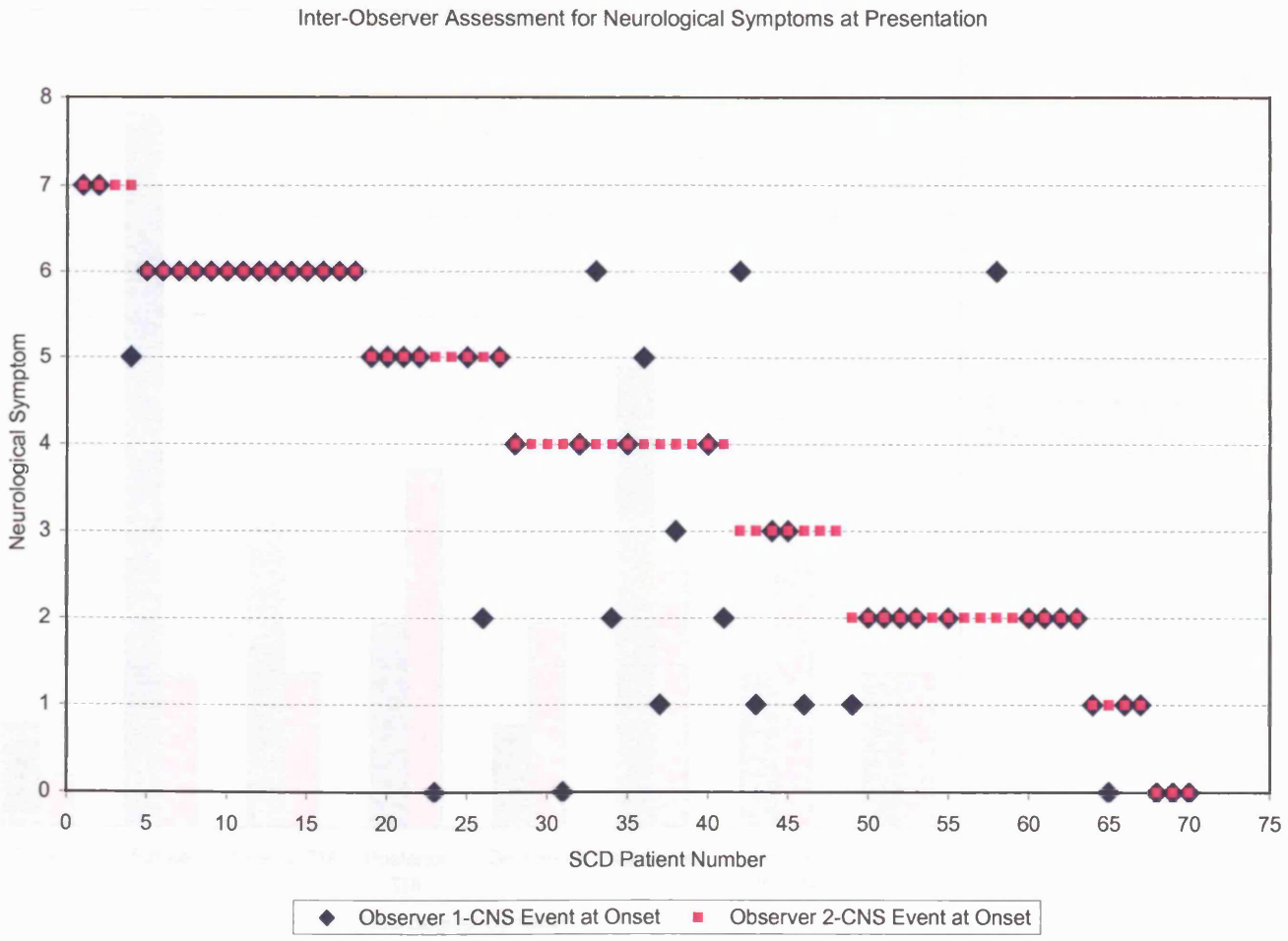


Figure 4.4. Inter-observer assessment in the short-(observer 1) and long-term (observer 2) of the neurological symptoms at presentation of the sickle cell patients for each symptom– cross- sectional data.

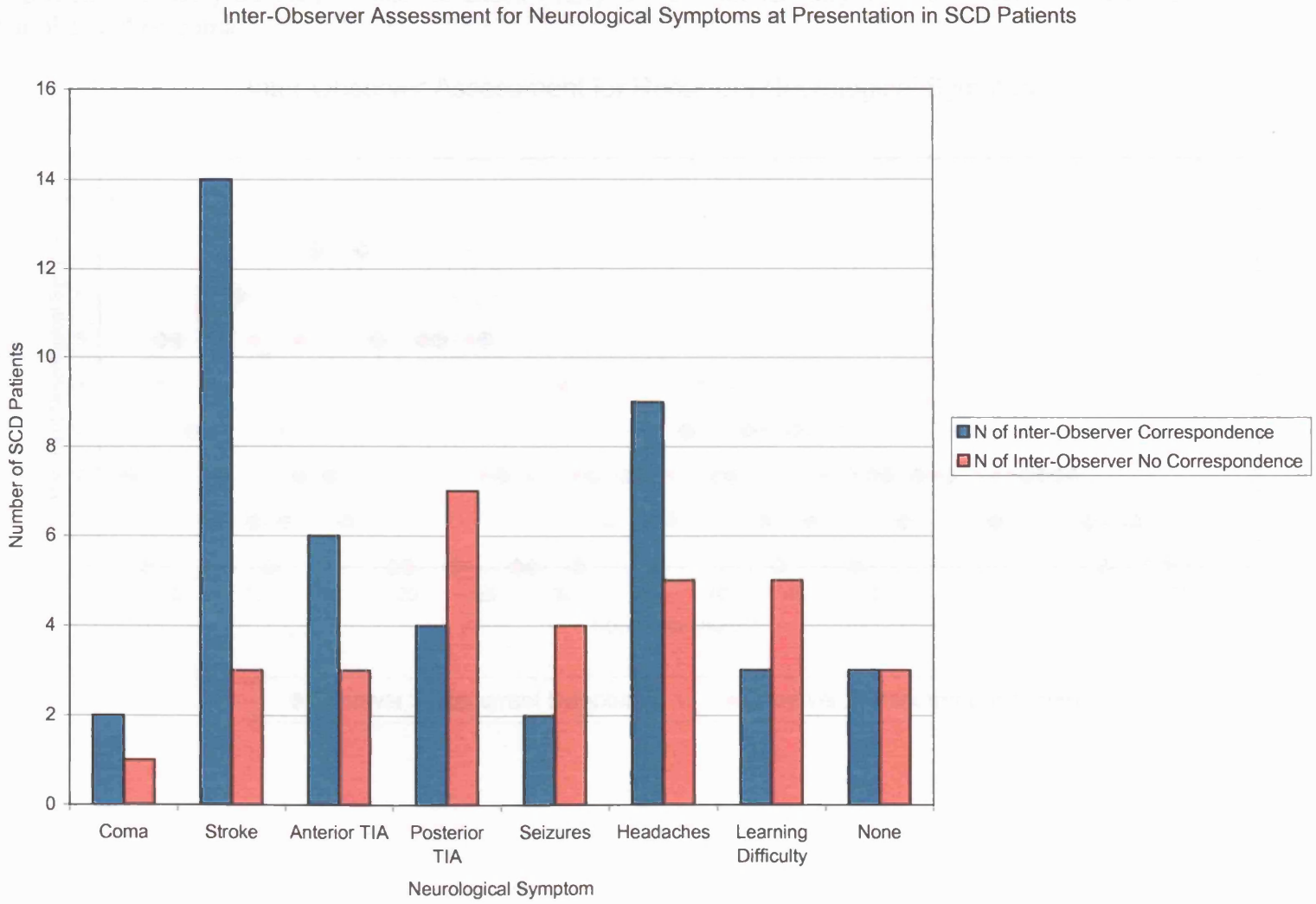


Figure 4.5. Inter-observer assessment in the short-(observer 1) and long-term (observer 2) of recurrent neurological symptoms of each sickle cell patient – cross- sectional data. Categories of symptoms: 0= none; 1= learning difficulty (cognitive symptom); 2= headaches; 3= seizures; 4= posterior territory transient ischaemic attack (TIA); 5= anterior territory TIA; 6= reversible ischaemic neurological deficit (RIND); 7= stroke; and 8= coma.

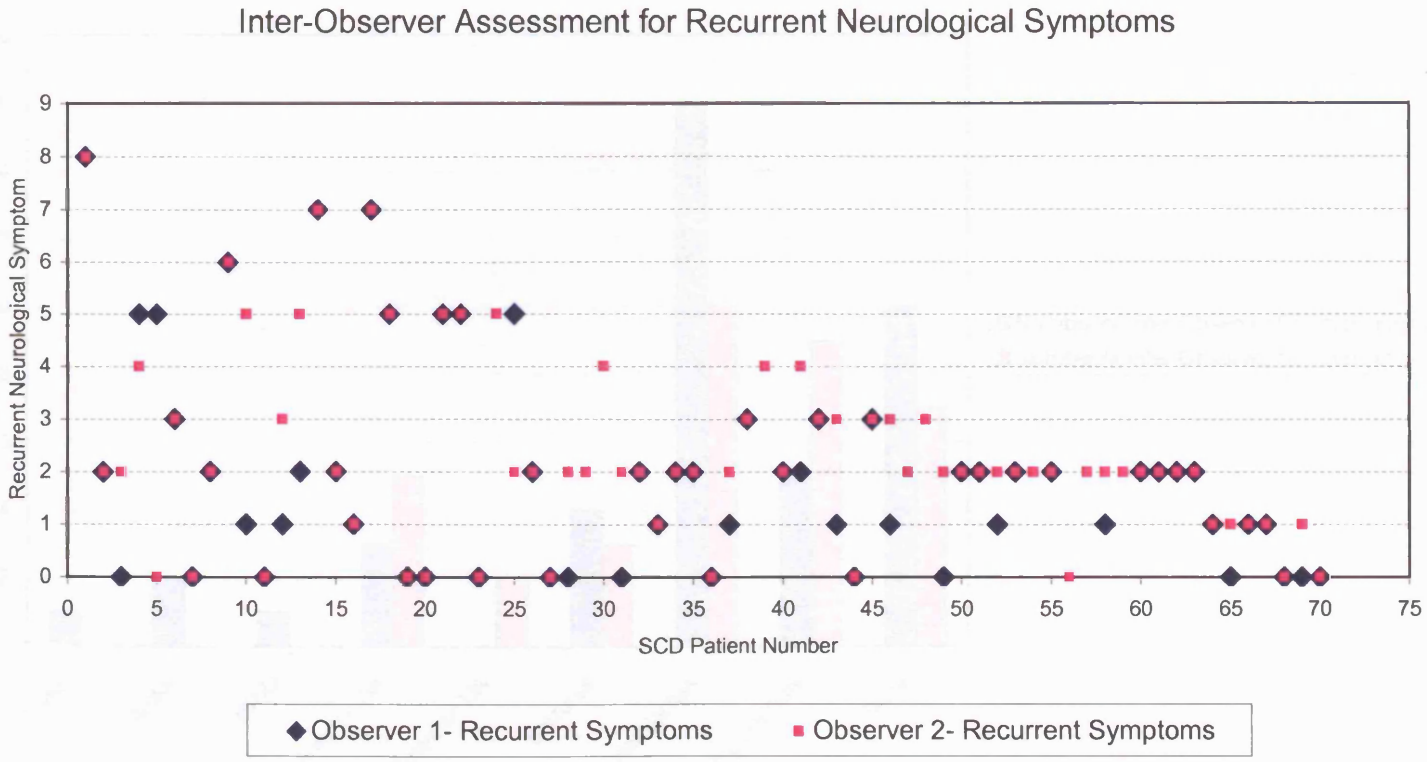
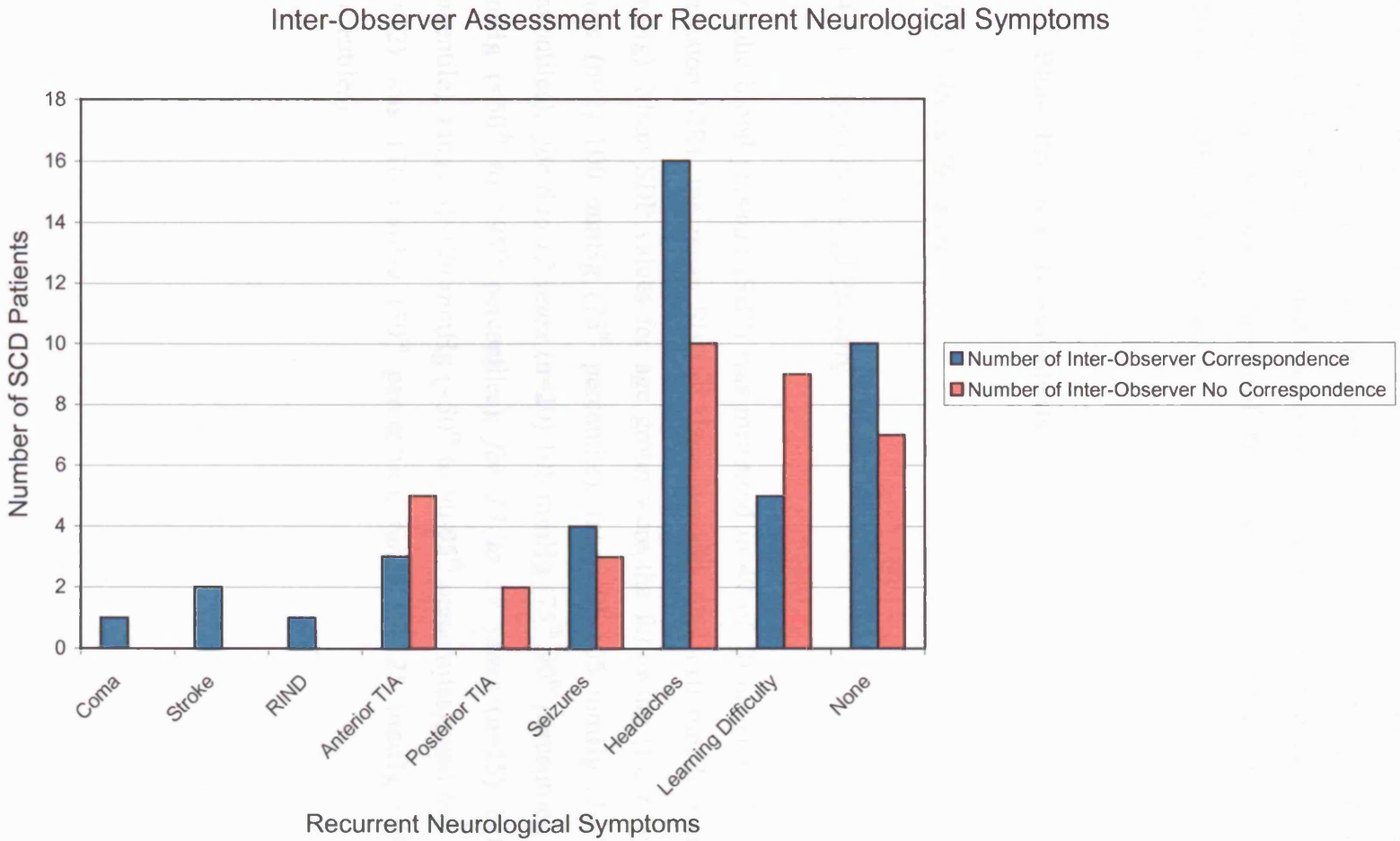


Figure 4.6. Inter-observer short- (observer 1) and long-term (observer 2) of recurrent neurological symptoms of the sickle cell patients for each symptom– cross- sectional data.



Therefore, for the purpose of the analysis of the clinical data in all the chapters, the descriptions of the neurological symptoms at presentation and recurrent symptoms of these sickle cell patients by the Consultant Paediatric Neurologist (observer 2) were chosen as she had followed and assessed these patients for a longer period of time, was actively involved in the treatment of their neurological complications, and was blinded to the results of the MR perfusion.

4.4.2. Blood Pressure Measurements

4.4.2.1. Blood Pressure

4.4.2.1.1. Systolic Blood Pressure

Systolic blood pressure (SBP) was measured in 49 of 70 patients who had successful perfusion MRI, the mean SBP of these patients was 110 mmHg (range 85 to 129 mmHg). Mean SBP values for age group were the following (figure 4.7): *for 3 to 5 years* (n=2) 100 mmHg (75^{th} percentile), range 95-105 mmHg (50-75^{th} to 75-90^{th} percentiles); *for 6 to 12 years* (n=20) 108 mmHg (75^{th}-90^{th} percentile), range 94 –128 mmHg (<50^{th} to >95^{th} percentiles); *for 13 to 17 years* (n=15) 111 mmHg (50^{th} percentile), range 85-129 mmHg (<50^{th} to 90-95^{th} percentiles); and *for 18 to 28 years* (n=12) was 116 mmHg (50^{th} percentile), range 98-125 mmHg (<50^{th} to 75-90^{th} percentiles).

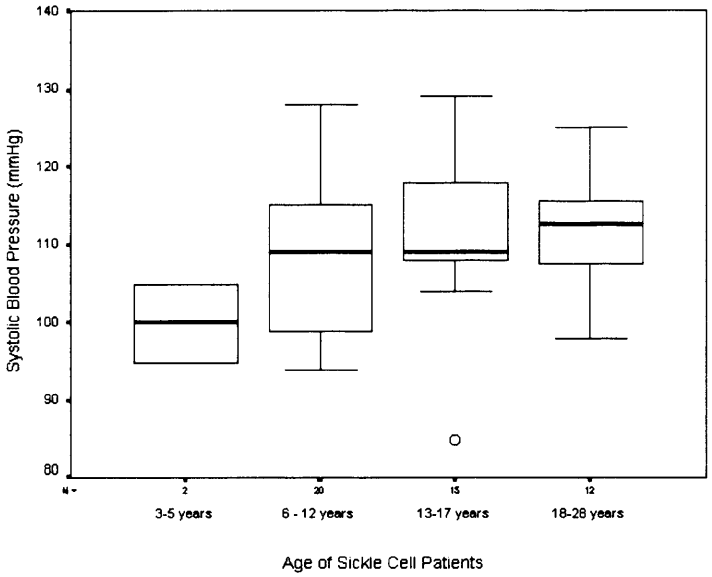


Figure 4.7. Systolic blood pressure measurements in relation to age.

Systolic blood pressure measurements of the sickle cell patients in relation to their neurological symptoms at presentation (figure 4.8) were the following: the mean SBP for patients who presented with coma (n=1) was 117 mmHg; for stroke (n=13) was 110 mmHg (range 95-128 mmHg); for anterior territory TIA (n=7) was 110 mmHg (range 100-122 mmHg); for posterior territory TIA (n=10) was 108 mmHg (range 85-129 mmHg); for seizures (n=3) was 107 mmHg (range 94-115 mmHg); headaches (n=11) was 112 mmHg (range 97-125 mmHg); for learning difficulty (n=2) was 104 mmHg (range 103-104 mmHg); and for asymptomatic patients (n=2) was 110 mmHg (range 108-112 mmHg). Table 4.1 shows the relationship between systolic blood pressure, neurological symptoms at presentation and SBP percentiles by age groups.

Neurological Symptoms	Age Group (years)	Patients (n)	Mean Systolic BP (mmHg)	Mean SBP Percentile for Age	SBP Range (mmHg)	SBP Percentile Range
Coma	6-12 y	1	117	75-90th	117	75-90th
Stroke	3-5 y	1	95	50th	95	50th
	6-12 y	3	108	75-90th	97-128	50- >95th
	13-17 y	4	112	50th	108-121	<50 – 75th
	18-28 y	5	113	50th	105-120	<50 – 75th
Anterior TIA	6-12 y	2	105	50-75th	100-109	50-75th
	13-17 y	2	119	50-75th	115-122	50-75th
	18-28 y	3	108	<50th	100-113	<50- 50th
Posterior TIA	6-12 y	3	107	50-75th	98-113	50 -90th
	13-17 y	6	110	<50th	85-129	<50 – 90th
	18-28 y	1	98	<50th	98	< 50th
Seizures	6-12 y	2	103	50-75th	94-111	<50 -90th
	18-28 y	1	115	<50th	115	<50th
Headaches	3-5 y	1	105	75-90th	105	75-90th
	6-12 y	5	112	75-90th	97-120	<50-95th
	13-17 y	3	107	<50th	104-110	<50th
	18-28 y	2	120	50-75th	115-125	50-75th
Learning Difficulty	6-12 y	2	104	50-75th	103-104	50-75th
Asymptomatic	6-12 y	2	110	75th	108-112	75-90th

Table 4.1. Relationship between systolic blood pressure (SBP), neurological symptoms at presentation and SBP percentiles by age groups in sickle cell patients.

Systolic blood pressure of the patients with SCD in relation to their recurrent neurological symptoms (figure 4.9) were the following: the mean SBP for patients who presented with coma (patient who presented with posterior leukoencephalopathy [PLKE]; n=1) was 117 mmHg; for stroke (n=2) was 114 mmHg (range 112-116 mmHg); for reversible ischaemic neurological deficit (RIND; n= 1) was 120 mmHg; for anterior territory TIA (n=6) was 112 mmHg (range 97-128 mmHg); for posterior territory TIA (n=1) was 109 mmHg; for seizures (n=5) was 108 mmHg (range 94-115 mmHg); headaches (n=21) was 110 mmHg (range 85-129 mmHg); for learning difficulty (n=5) was 104 mmHg (range 98-108 mmHg); and for asymptomatic patients (n=7) was 106 mmHg (range 95-115 mmHg). Table 4.2 shows the relationship between systolic blood pressure, recurrent neurological symptoms and SBP percentiles by age groups.

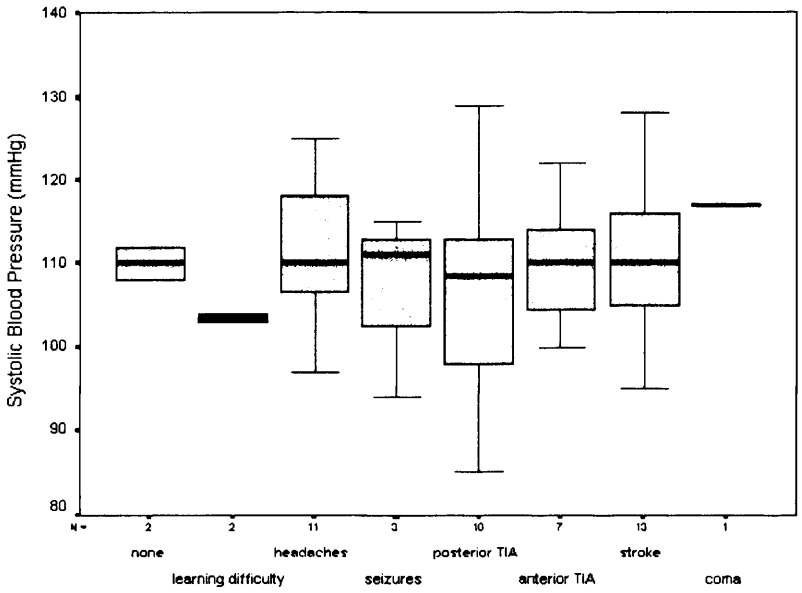


Figure 4.8. Systolic blood pressure measurements in relation to neurological symptoms at presentation in sickle cell patients. TIA: transient ischaemic attack.

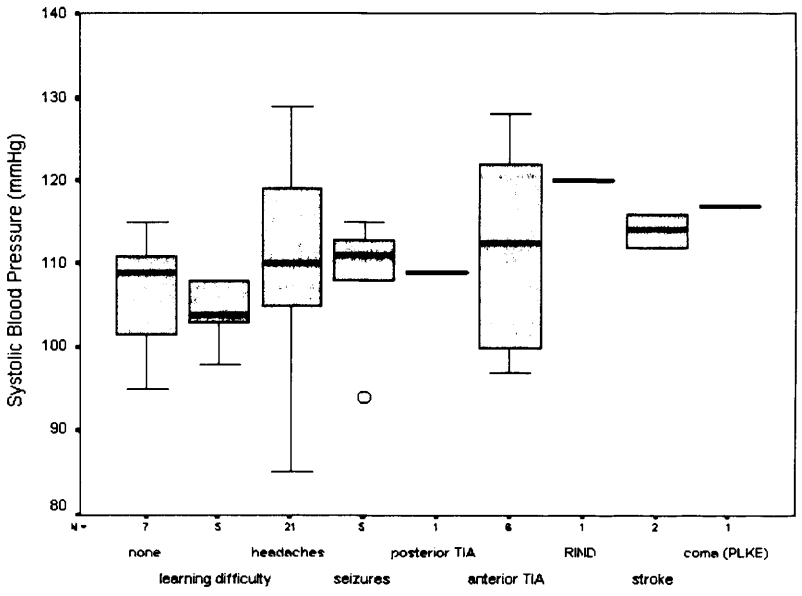


Figure 4.9. Systolic blood pressure measurements in relation to recurrent neurological symptoms in sickle cell patients. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy

Recurrent Neurological Symptoms	Age Group	Patients (n)	Mean Systolic BP (mmHg)	Mean SBP Percentile for Age	SBP Range (mmHg)	SBP Percentile Range
Coma	6-12 y	1	117	75-90th	117	75-90th
Stroke	18-28 y	2	114	50th	112-116	50th
RIND	18-28 y	1	120	75th	120	75th
Anterior TIA	6-12 y	3	108	75-90th	97-128	<50->95th
	13-17 y	2	117	50-75th	112-122	<50-75th
	18-28 y	1	113	50th	113	50th
Posterior TIA	13-17 y	1	109	<50th	109	<50th
Seizures	6-12 y	3	106	75th	94-113	<50- 90th
	13-17 y	1	108	<50th	108	<50th
	18-28 y	1	115	50-75th	115	50-75th
Headaches	3-5 y	1	105	75-90th	105	75-90th
	6-12 y	6	112	75th	97-120	<75-90th
	13-17 y	9	110	<50-50th	85-129	<50-90th
	18-28 y	5	110	<50-50th	98-125	<50-75th
Learning Difficulty	6-12 y	4	103	50th	98-108	<50-75th
	13-17 y	1	108	<50th	108	<50th
Asymptomatic	3-5 y	1	95	50th	95	50th
	6-12 y	3	106	50-75th	98-112	<50-90th
	13-17 y	1	115	50-75th	115	50-75th
	18-28 y	2	108	<50th	105-110	<50-50th

Table 4.2. Relationship between systolic blood pressure (SBP), recurrent neurological symptoms and systolic blood pressure percentiles by age groups in sickle cell patients.

4.4.2.1.2. Diastolic Blood Pressure

Diastolic blood pressure (DBP) was measured in 49 of 70 patients who had successful perfusion MRI. The mean DBP of these patients was 58 mmHg (range 30 to 81 mmHg). Mean DBP values for age group were the following (figure 4.10): *for 3 to 5 years* (n=2) 46 mmHg (<50th percentile), range 34-57 mmHg (<50th to 50th percentile); *for 6 to 12 years* (n=20) 59 mmHg (<50th -50 percentile), range 33-81 mmHg (<50th to >95th percentile); *for 13 to 17 years* (n=15) 56 mmHg (<50th percentile), range 31-76 mmHg

(<50th to 75-90th percentile); and *for 18 to 28 years* (n=12) was 59 mmHg (<50th percentile), range 30-80 mmHg (<50th to 90th percentile).

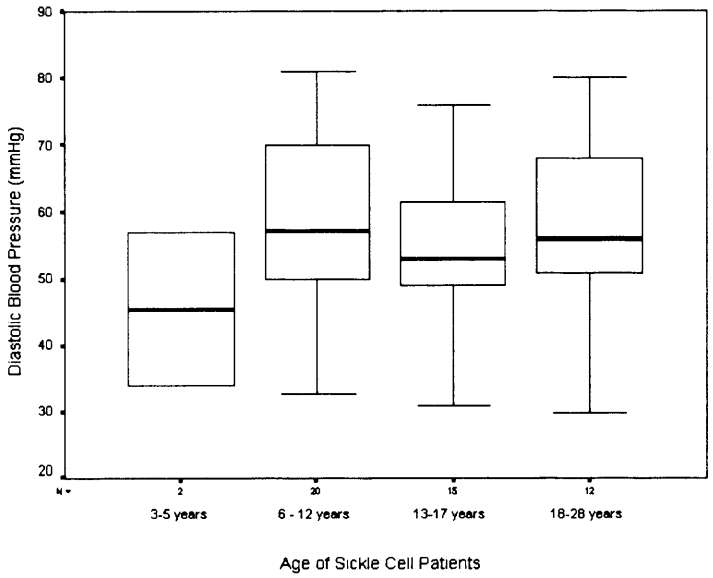


Figure 4.10. Diastolic blood pressure measurements in relation to age.

Diastolic blood pressure measurements of the sickle cell patients in relation to their neurological symptoms at presentation (figure 4.11) were the following: the mean DBP for patients who presented with coma (n= 1) was 55 mmHg; for stroke (n=13) was 56 mmHg (range 31 to 80 mmHg); for anterior territory TIA (n=7) was 53 mmHg (range 30 to 76 mmHg); for posterior territory TIA (n=10) was 62 mmHg (range 49 to 79 mmHg); for seizures (n=3) was 68 mmHg (range 58 to 81 mmHg); headaches (n=11) was 56 mmHg (range 33 to 80 mmHg); for learning difficulty (n=2) was 61 mmHg (range 51 to 71 mmHg); and for asymptomatic patients (n=2) was 50 mmHg (range 48 to 50 mmHg). Table 4.3 shows the relationship between diastolic blood pressure, neurological symptoms at presentation and DBP percentiles by age groups.

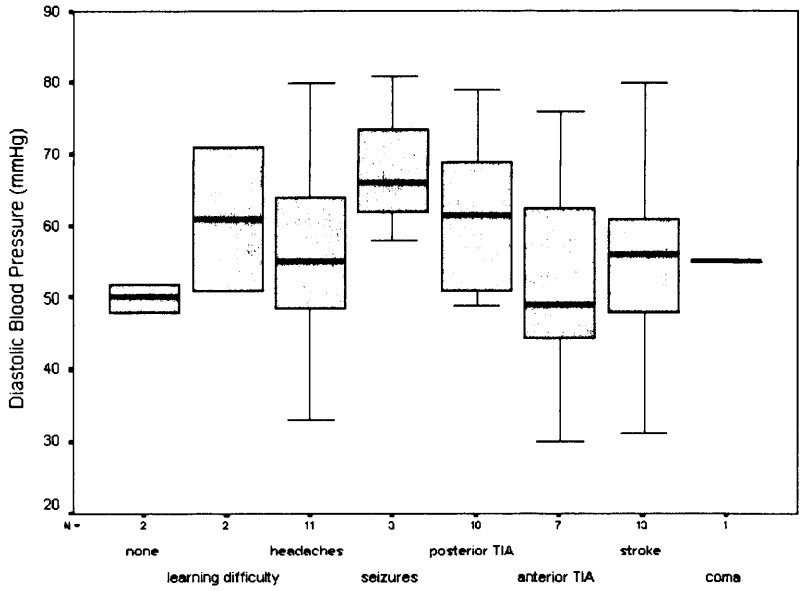


Figure 4.11. Diastolic blood pressure measurements in relation to neurological symptoms at presentation in sickle cell patients. TIA: transient ischaemic attack

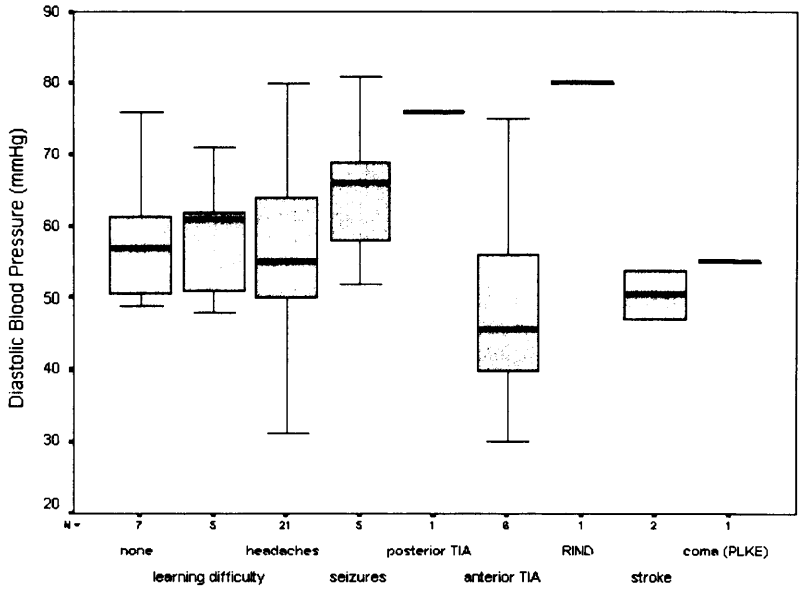


Figure 4.12. Diastolic blood pressure measurements in relation to recurrent neurological symptoms in sickle cell patients. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy

Neurological Symptoms	Age Group	Patients (n)	Mean Diastolic BP (mmHg)	Mean DBP Percentile for Age	DBP Range (mmHg)	DBP Percentile Range
Coma	6-12 y	1	55	<50th	55	<50th
Stroke	3-5 y	1	57	50-75th	57	50-75th
	6-12 y	3	53	<50th	42-61	<50-50th
	13-17 y	4	52	<50th	31-75	<50 – 75th
	18-28 y	5	61	<50th	47-80	<50 – 90th
Anterior TIA	6-12 y	2	58	<50-50th	40-76	<50-90th
	13-17 y	2	49	<50th	49-49	<50th
	18-28 y	3	52	<50th	30-70	<50- 75th
Posterior TIA	6-12 y	3	66	75th	49-79	<50 -95th
	13-17 y	6	62	<50th	51-76	<50 – 90th
	18-28 y	1	50	<50th	50	< 50th
Seizures	6-12 y	2	70	75-90th	58-81	<50 - >95th
	18-28 y	1	66	50th	66	50th
Headaches	3-5 y	1	34	<50th	34	<50th
	6-12 y	5	59	<50th	33-80	<50->95th
	13-17 y	3	53	<50th	45-61	50th
	18-28 y	2	66	50th	52-79	<50-90th
Learning Difficulty	6-12 y	2	61	50th	51-71	<50-90th
Asymptomatic	6-12 y	2	50	<50th	48-52	<50th

Table 4.3. Relationship between diastolic blood pressure (DBP), neurological symptoms at presentation and DBP percentiles by age groups in sickle cell patients.

Diastolic blood pressure of the patients with SCD in relation to recurrent neurological symptoms (figure 4.12) were the following: the mean DBP for patients who presented with coma (patient who presented with PLKE; n=1) was 55 mmHg; for stroke (n=2) was 51 mmHg (range 47 to 54 mmHg); for reversible ischaemic neurological deficit (RIND; n=1) was 80 mmHg; for anterior territory TIA (n=6) was mmHg (range mmHg); for posterior territory TIA (n=1) was 76 mmHg ; for seizures (n=5) was 65 mmHg (range 52 to 81 mmHg); headaches (n=21) was 57 mmHg (range 31 to 80 mmHg); for learning difficulty (n=5) was 59 mmHg (range 48 to 71 mmHg); and for asymptomatic patients (n=7) was 58 mmHg (range 49 to 76 mmHg). Table 4.4 shows the relationship among diastolic blood pressure, recurrent neurological symptoms and DBP percentiles by age groups.

Recurrent Neurological Symptoms	Age Group	Patients (n)	Mean Diastolic BP (mmHg)	Mean DBP Percentile for Age	DBP Range (mmHg)	DBP Percentile Range
Coma	6-12 y	1	55	<50th	55	<50th
Stroke	18-28 y	2	51	<50th	47-54	<50th
RIND	18-28 y	1	80	90th	80	90th
Anterior TIA	6-12 y	3	46	<50th	40-56	<50th
	13-17 y	2	62	<50th	49-75	<50-75th
	18-28 y	1	30	<50th	30	<50th
Posterior TIA	13-17 y	1	76	75-90th	76	75-90th
Seizures	6-12 y	3	69	75th	58-81	<50- 95th
	13-17 y	1	52	<50th	52	<50th
	18-28 y	1	66	50th	66	50th
Headaches	3-5 y	1	34	<50th	34	<50th
	6-12 y	6	63	50-75th	33-80	<50-95th
	13-17 y	9	53	<50th	31-64	<50-50th
	18-28 y	5	61	<50th	50-79	<50-90th
Learning Difficulty	6-12 y	4	58	<50-50th	48-71	<50-90th
	13-17 y	1	62	<50th	62	<50th
Asymptomatic	3-5 y	1	57	50-75th	57	50-75th
	6-12 y	3	59	<50th	49-76	<50-90th
	13-17 y	1	49	<50th	49	<50th
	18-28 y	2	62	<50th	57-66	<50-50th

Table 4.4. Relationship between diastolic blood pressure (DBP), recurrent neurological symptoms and DBP percentiles by age groups in sickle cell patients.

4.4.2.1.3. Mean Arterial Blood Pressure

Mean arterial blood pressure (MAP) was measured in 49 of 70 patients who had successful perfusion MRI, and the mean MAP of these patients was 76 mmHg (range 55 to 97 mmHg). Mean MAP values for age group were the following (figure 4.13): for *3 to 5 years* (n=2) 64 mmHg (range 58-70 mmHg); *for 6 to 12 years* (n=20) 77 mmHg (range 55-95 mmHg); *for 13 to 17 years* (n=15) 75 mmHg (range 61-90 mmHg); and *for 18 to 28 years* (n=12) 77 mmHg (range 58-97 mmHg).

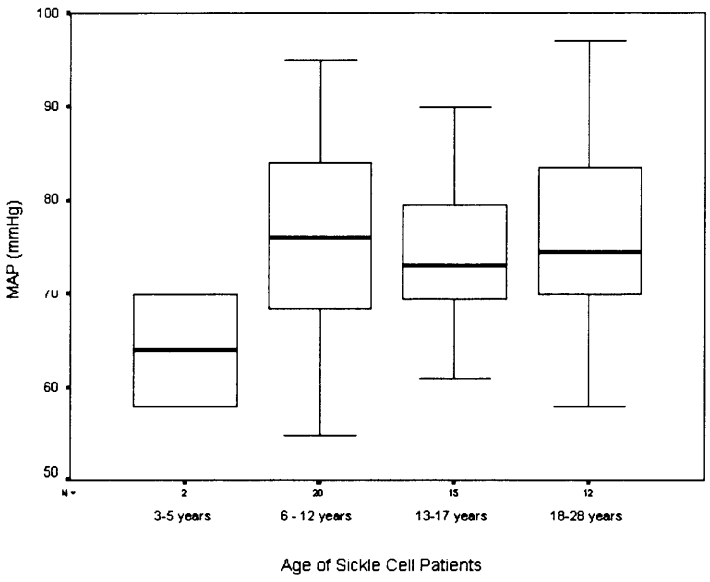


Figure 4.13. Mean arterial blood pressure (MAP) pressure measurements in relation to the age of the sickle cell patients.

MAPs of the sickle cell patients in relation with their neurological symptoms at presentation (see figure 4.14) were the following: the mean MAP for patients who presented with coma (n=1) was 76 mmHg; for stroke (n=13) was 75 mmHg (range 61 to 93 mmHg); for anterior territory TIA (n=7) was 73 mmHg (range 58 to 90 mmHg); for posterior territory TIA (n=10) was 78 mmHg (range 66 to 92 mmHg); for seizures (n=3) was 84 mmHg (range 72 to 95 mmHg); headaches (n=11) was 76 mmHg (range 55 to 97 mmHg); for learning difficulty (n=2) was 77 mmHg (range 69 to 84 mmHg); and for asymptomatic patients (n=2) was 71 mmHg (range 68 to 73 mmHg).

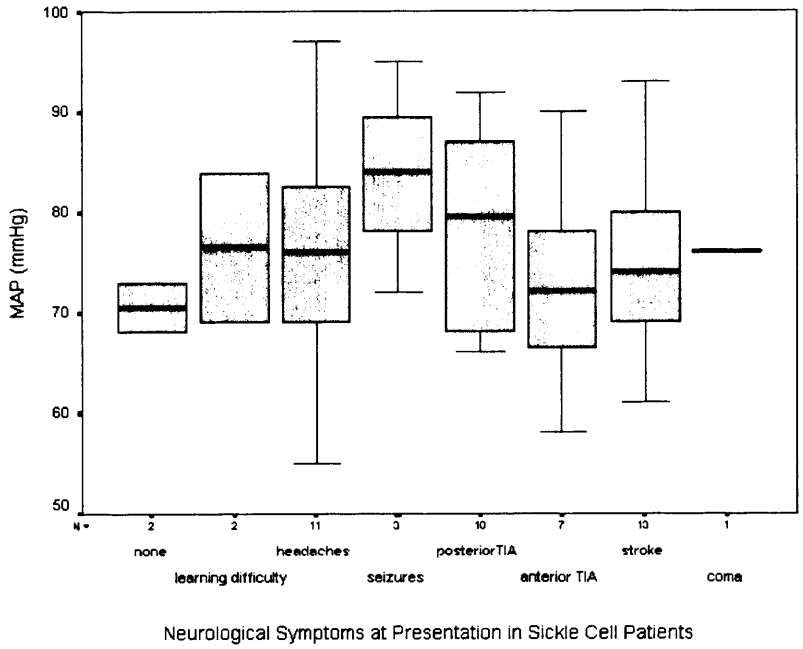


Figure 4.14. Mean arterial pressure (MAP) in relation to the neurological symptoms at presentation in sickle cell patients. TIA: transient ischaemic attack

MAPs of the patients with SCD in relation to recurrent neurological symptoms (figure 4.15) were the following: the mean MAP for patients who presented with coma (patient who presented with PLKE; n=1) was 76 mmHg; for stroke (n=2) was 71 mmHg (range 68 to 74 mmHg); for reversible ischaemic neurological deficit (RIND; n=1) was 93 mmHg; for anterior territory TIA (n=6) was 71 mmHg (range 58 to 90 mmHg); for posterior territory TIA (n=1) was 90 mmHg; for seizures (n=5) was 81 mmHg (range 72 to 95 mmHg); headaches (n=21) was 75 mmHg (range 55 to 97 mmHg); for learning difficulty (n=5) was 75 mmHg (range 68 to 84 mmHg); and for asymptomatic patients (n=7) was 75 mmHg (range 66 to 90 mmHg).

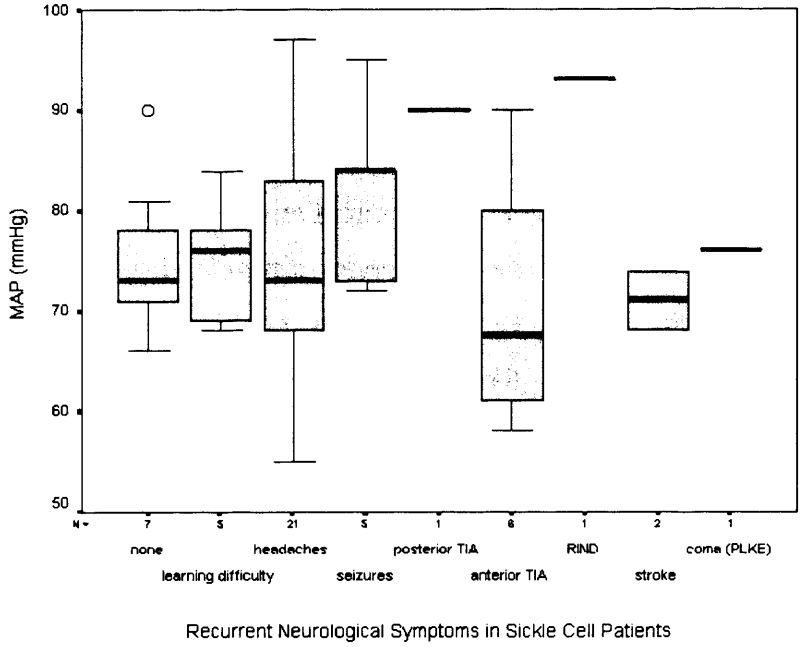


Figure 4.15. Mean arterial pressure (MAP) in relation to the recurrent neurological symptoms in sickle cell patients. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy

4.4.2.1.4. Effect of Blood Pressure on Neurological Symptoms at Presentation and Recurrent Neurological Symptoms in Sickle Cell Patients

There were no significant associations between systolic (SP), diastolic (DP) and mean arterial (MAP) blood pressure values and the presence of neurological symptoms at presentation in sickle cell patients (p=0.9, p=0.4 and p=0.5 for SP, DP and MAP respectively, logistic regression). In addition, presence of neurological symptoms at presentation related more specifically to a vasculopathy/ischaemia cause, such as stroke, TIA and seizures in SCD (Adams et al 1994, Prengler et al 2002), were also not significantly associated with higher BP values (p=0.7, p=0.6 and p=0.7 for SP, DP and MAP respectively, logistic regression).

There were no significant differences in mean blood pressure values among the different neurological symptoms at presentation (p=0.9, p=0.7 and p=0.9 for SP, DP and MAP respectively, one-way ANOVA). Post-hoc tests were not performed because of the small number of patients for some symptoms.

Recurrent neurological symptoms (presence or not of symptoms) were not significantly associated with higher blood pressure values (p=0.3, p=0.9 and p=0.9 for SP, DP and MAP respectively, logistic regression). Moreover, recurrent neurological symptoms more associated with vasculopathy/ischaemia (stroke, TIA, RIND and seizures) were also not significantly associated with higher blood pressure (p=0.4, p=0.8, p=0.6 for SP, DP and MAP respectively, logistic regression). Similarly, there were no significant differences between mean blood pressure values among the different recurrent neurological symptoms (p=0.7, p=0.3, p=0.5 for SP, DP and MAP respectively, one–way ANOVA). Post-hoc tests were also not performed because the small number of patients for some symptoms.

Post-hoc analysis of the blood pressure values was done in order to examine if there were differences in BP depending on the age of the patients with SCD and neurological symptoms. There were no significant differences between the mean SP, DP and MAP among the age groups (p=0.3, p=0.5 and 0.4 for SP, DP and MAP respectively, Tukey's test).

However, sickle cell patients studied between 6 and 12 years had higher percentiles for mean systolic and/or diastolic blood pressures (75-90th percentile) for some neurological symptoms at presentation (coma and stroke) and recurrent symptoms (seizures, posterior TIA) compared to other age groups (tables 4.1-4.4). Similarly, the percentile range was higher for this age group (up to 90th or >95th percentiles) for systolic pressure in some neurological symptoms at presentation (coma, stroke, posterior TIA and headaches) and in some recurrent symptoms (such as coma, anterior TIA and headaches). There were also similar very high percentile range (up to 90th or >95th percentiles) diastolic pressures in patients with some neurological symptoms (such as anterior and posterior TIA, seizures, headaches and learning difficulty), and in patients with some recurrent symptoms (such as seizures and headaches).

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4.4.3. Haematological Parameters

The available haematological data of the patients (closest to the date of the investigations) was collected from clinical records. The blood tests were done with a mean of 5 months (range 0-2.9 years [between 2.9 years before and 1.6 years after])

from the date of the magnetic resonance and transcranial Doppler ultrasound studies. The haematological data analysed were haemoglobin, haemoglobin S%, white cell count and platelets.

Other haematological data were also collected from clinical records such as neutrophils, lymphocytes, reticulocytes, foetal haemoglobin, ferritin, bilirubin, calcium and magnesium; however, the numbers of these data were too small for analysis, therefore these haematological and biochemical parameters were excluded from this study.

4.4.3.1. Haemoglobin

Haemoglobin (Hb) level: Haemoglobin level data were collected from 65 patients. Mean Hb level was 8.9 g/dL, with a range from 5.7 to 12.9 g/dL. Table 4.5 shows the Hb levels by age and their normal values (Nelson et al 1996). As shown in the table, the mean Hb values for sickle cell patients were all below the normal values for age.

Patients Age	**Hb (g/dL) Mean**	**Hb (g/dL) Range**	***Normal Values (g/dL)***
6-18 months (n=3)	8.7	8.2 - 9.6	*9 -14*
2-5 years (n=1)	6.9	6.9	*9 -14*
6-12 years (n=28)	8.9	5.7 – 12.9	*11.5-15.5*
13-17 years (n=20)	8.9	6.6 – 11	*Male: 13-16 Female: 12-16*
18-28 years (n=13)	8.9	6.1 – 10.7	*Male: 13.5-17.5 Female: 12-16*

Table 4.5. Haemoglobin level in sickle cell patients in relation to age groups.

In relation to haemoglobin levels, there was a trend for an association between haemoglobin levels and central nervous system events at presentation (p=0.1, one-way ANOVA; figure 4.16). As shown in table 4.6, patients without symptoms had the lower mean Hb levels (Hb 7.7.g/d/L), whereas the sickle cell patients with seizures, stroke and

coma had the highest mean Hb levels (Hb 9.2.-9.7 g/dL); 13 patients with stroke and 2 patients who presented with coma were on chronic blood transfusion, but none of the 7 patients with seizures (only 1 of 7 seizure patients received transfusion acutely).

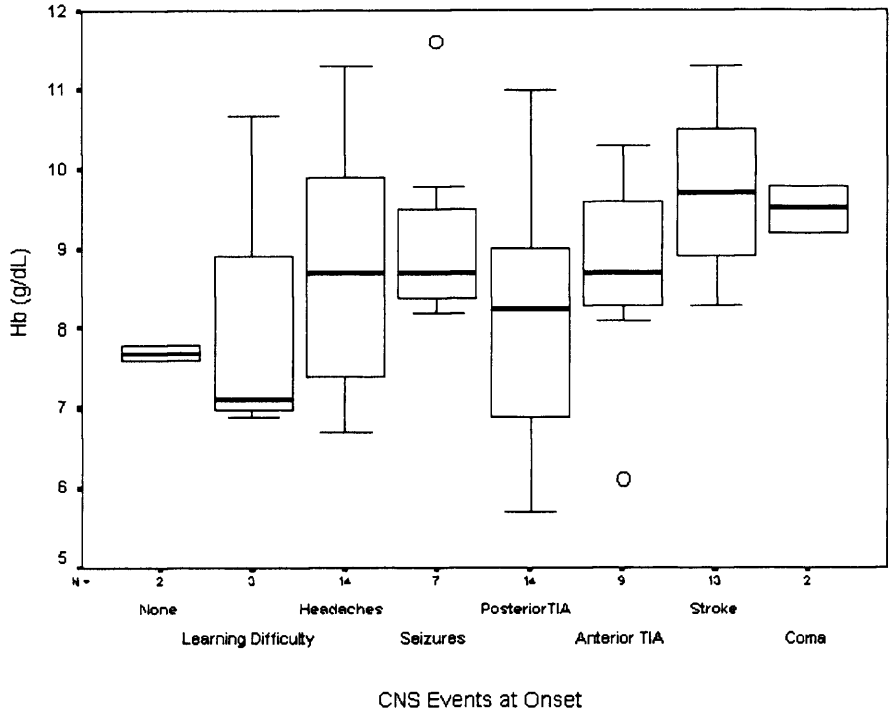


Figure 4.16. Haemoglobin level in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

CNS Events (n = Patients)	Hb (g/dL) Mean	Hb (g/dL) Range
No Symptoms (n=2)	7.7	7.6 - 7.8
Learning Difficulty (n=3)	8.2	6.9 - 10.7
Headaches (n=14)	8.6	6.7 - 11.3
Seizures (n=7)	9.2	8.2 – 11.6
Posterior TIA (n=14)	8.2	5.7 - 11
Anterior TIA (n=9)	8.7	6.1 – 10.3
Stroke (n=13)	9.7	8.3 – 11.3
Coma (n=2)	9.5	9.2 – 9.8

Table 4.6. Haemoglobin level in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

There was no association between the mean levels of haemoglobin and recurrent neurological symptoms (p=0.9, one-way ANOVA, figure 4.17 and table 4.7). Patients with recurrent posterior territory TIAs had the lowest mean Hb values (Hb 7.7 g.dL) and the patients with stroke had the highest (Hb 9.5 g/dL); again the majority were on chronic transfusion programmes.

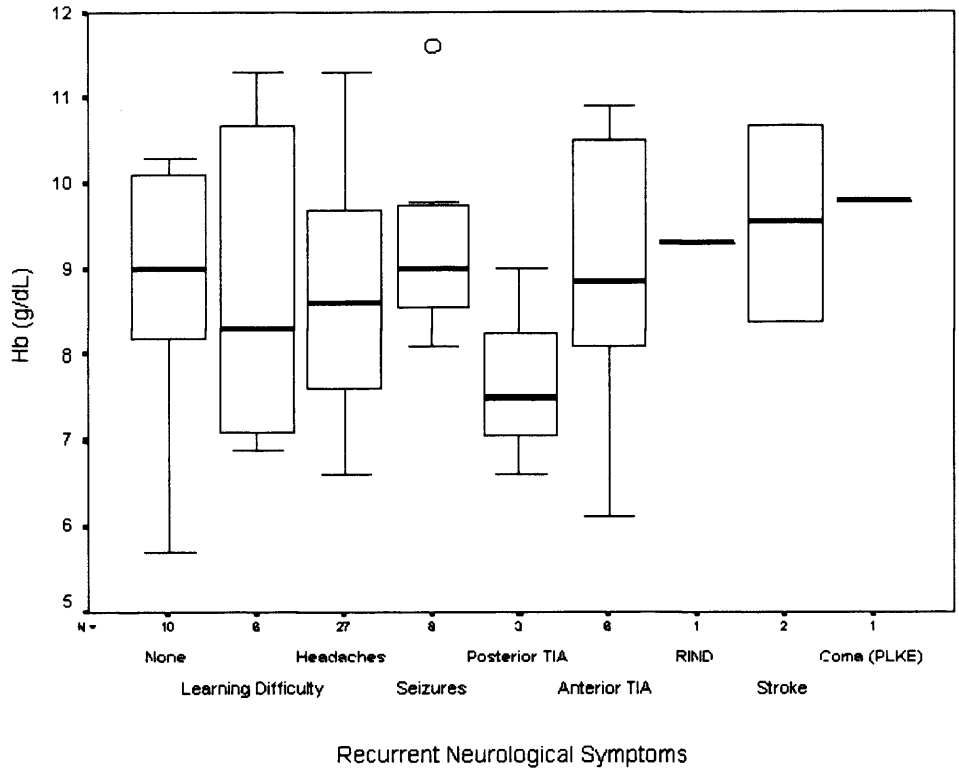


Figure 4.17. Haemoglobin level (g/dL) in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Recurrent Symptom (n =Patients)	Hb (g/dL) Mean	Hb (g/dL) Range
No Symptoms (n=10)	8.8	5.7 – 10.3
Learning Difficulty (n=6)	8.8	6.9 – 11.3
Headaches (n=27)	8.7	6.6 – 11.3
Seizures (n=8)	9.3	8.1 – 11.6
Posterior TIA (n=3)	7.7	6.6 – 9
Anterior TIA (n=6)	8.9	6.1 – 10.9
RIND (n=1)	9.3	9.3
Stroke (n=2)	9.5	8.4 – 10.7
Coma (PLKE) (n=1)	9.8	9.8

Table 4.7. Haemoglobin level (g/dL) in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Abnormal TCD was significantly associated with higher levels of Hb (p=0.019, logistic regression; figure 4.18). There was a trend for an association between abnormal MRI (presence of cerebral infarction) and higher Hb levels (p=0.1, logistic regression; figure 4.19). There was no significant association between Hb level and abnormal MRA

(p=0.9). Hb levels were higher in those with abnormal perfusion MRI, although this was not statistically significant (p=0.2, logistic regression; figure 4.20). Table 4.8 shows the mean and range of Hb level for each investigation.

Investigations (n=pats)	Normal Investigation Mean Hb (g/dL) (range)	Abnormal Investigation Mean Hb (g/dL) (range)	P value
MRI * (n=64)	8.6 (5.7 -11.6)	9.1 (6.1 – 11.3)	p=0.1
MRA (n=63)	8.7 (5.7 – 11.6)	8.9 (6.1 - 11.3)	p=0.9
TCD ** (n=61)	8.7 (6.6 – 11.6)	8.9 (5.7 – 11.3)	p=0.019
Perfusion MRI (n=64)	8.5 (5.7 – 11.3)	9 (6.1 – 11.6)	p=0.2

Table 4.8. Haemoglobin levels in relation to whether MR and TCD studies are normal or abnormal. *p=0.1; ** p=<0.05.

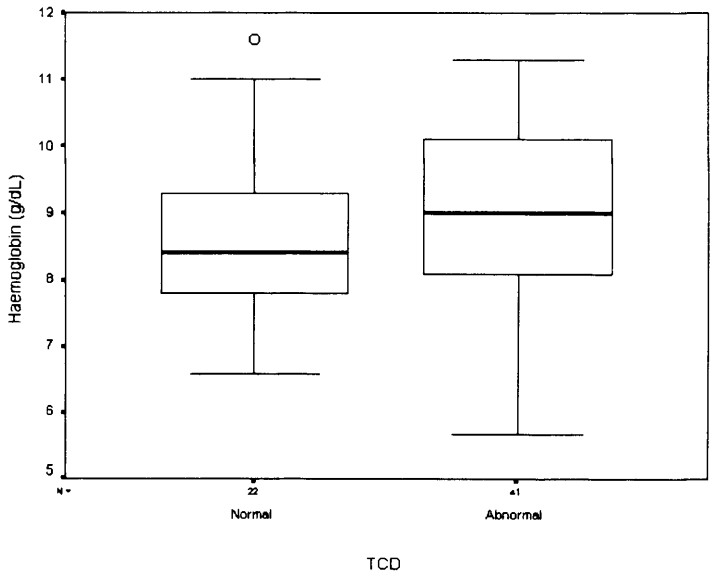


Figure 4.18. TCD (normal or abnormal) and haemoglobin level (p=0.019)

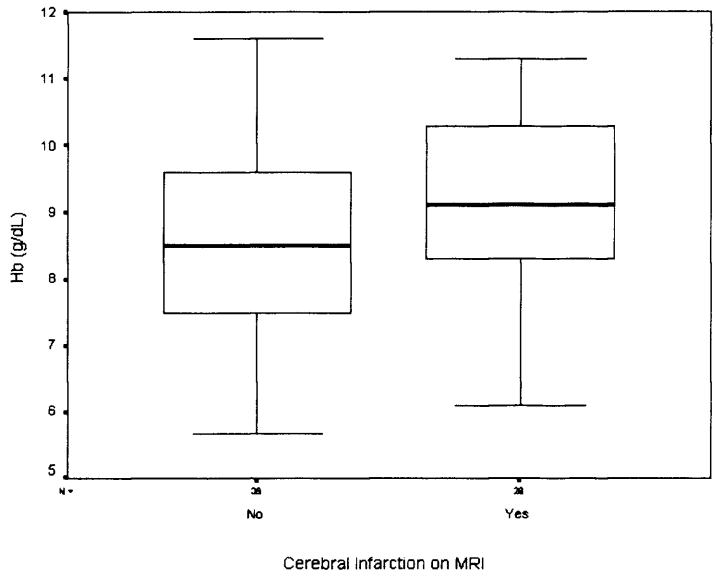


Figure 4.19. MRI (abnormal= presence of cerebral infarction) and haemoglobin level (p=0.1).

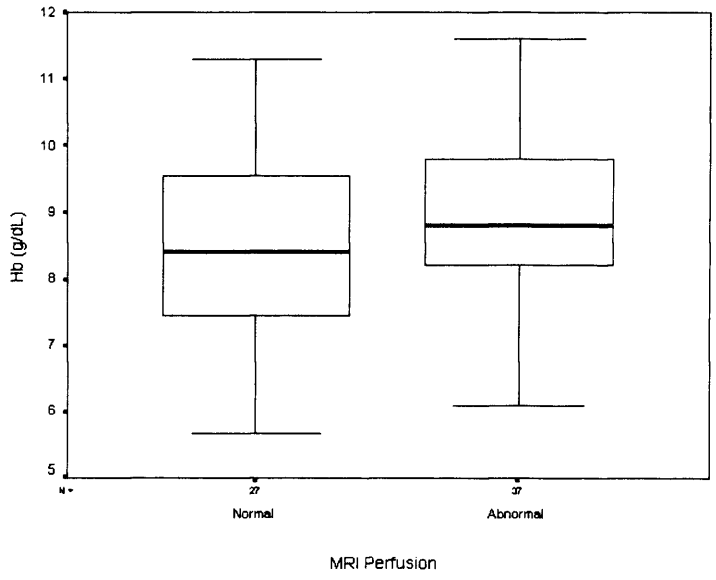


Figure 4.20. Perfusion MRI (normal or abnormal perfusion) and haemoglobin level (p=0.2).

4.4.3.2. Haemoglobin S

Haemoglobin S% (HbS%): The available haemoglobin S% level data were collected from 40 patients. Median HbS% level was 58%, with a range from 6 to 91%.

The relationships between the mean and range of HbS% and each of the central nervous system events at onset and recurrent neurological symptoms are shown in tables 4.9 and 4.10. There was a significant difference between the means of the patients' levels of HbS% and their CNS symptoms at onset (p=0.002, one-way ANOVA; figure 4.21); patients with anterior TIA, stroke and coma had lower mean HbS% values than the patients with other symptoms (patients on blood transfusion). However there was no significance association between the means of the patients' HbS% and their recurrent neurological symptoms (p=0.2, one-way ANOVA, figure 4.22), although patients with the same symptoms described above also had lower HbS% values. Post-hoc analysis was not performed because the small numbers of patients for each symptom (at onset or recurrence).

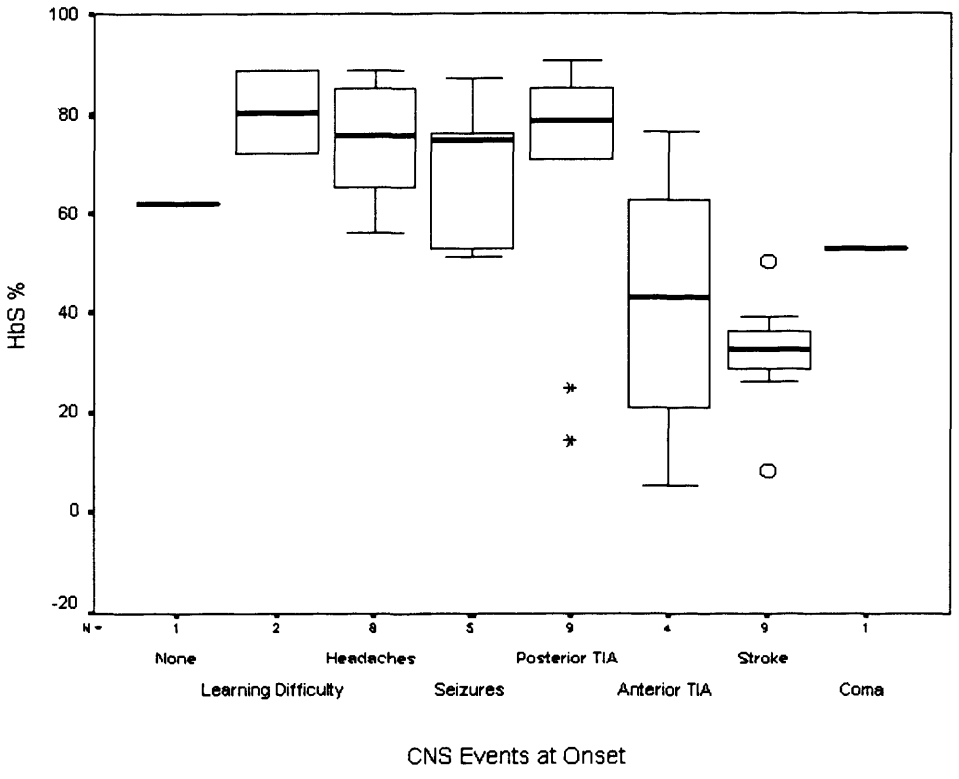


Figure 4.21. Haemoglobin S% levels in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

CNS Events (n = Patients)	HbS% Mean	HbS% Range
No Symptoms (n=1)	62%	62%
Learning Difficulty (n=2)	80%	72-89%
Headaches (n=8)	75%	56-88%
Seizures(n=5)	68%	51-87%
Posterior TIA(n=9)	68%	15-91%
Anterior TIA (n=4)	42%	6-76%
Stroke (n=9)	32%	8-50%
Coma (n=1)	53%	53%

Table 4.9. Haemoglobin S % levels in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

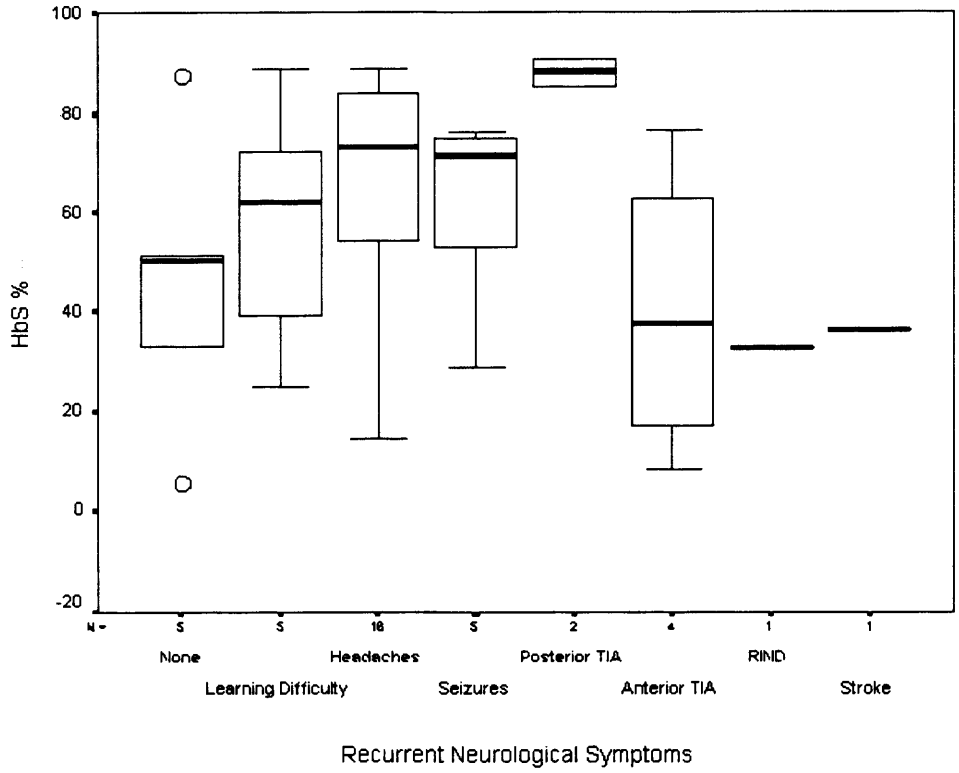


Figure 4.22. Haemoglobin S% levels in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Recurrent Symptom (n = Patients)	Hb S % Mean	Hb S% Range
No Symptoms (n=5)	45%	6 – 87%
Learning Difficulty (n=5)	57%	25 – 89%
Headaches (n=16)	66%	15 – 88%
Seizures (n=5)	61%	29 -76%
Posterior TIA (n=2)	88%	85 – 91%
Anterior TIA (n=4)	40%	8 – 76%
RIND (n=1)	33%	33%
Stroke (n=1)	36%	36%

Table 4.10. Haemoglobin S% levels in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Decreased levels of HbS% were significantly associated with abnormal MRA (p=0.001, logistic regression; figure 4.23) and abnormal perfusion MRI (p=0.002; figure 4.24).

There was a trend for an association between decreased HbS% and abnormal TCD (p=0.06; figure 4.25). There was no association with the presence of cerebral infarction on MRI (p=0.8). Table 4.11 shows the mean and range of HbS% in relation to each investigation.

Investigations (n=pats)	Normal HbS% Mean (Range)	Abnormal HbS% Mean (Range)	P value
MRI (n=39)	60% (6-91%)	33% (29 -36%)	p=0.8
MRA (n=39)	75% (37 -88%)	44% (6-91%)	p=0.001**
TCD (n=39)	71% (50-89%)	53% (6-91%)	p=0.06*
Perfusion MRI (n=39)	73% (6-91%)	45% (8-76%)	p=0.002**

Table 4.11. HbS% levels in relation to whether MR and TCD investigations are normal or abnormal in sickle cell patients. * Trend for an association; ** p<0.05.

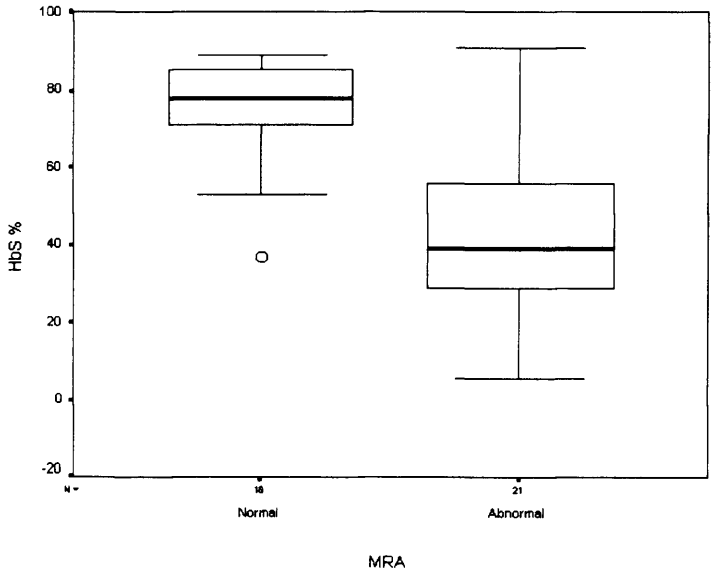


Figure 4.23. Relationship between abnormal MRA and HbS% values (p=0.001).

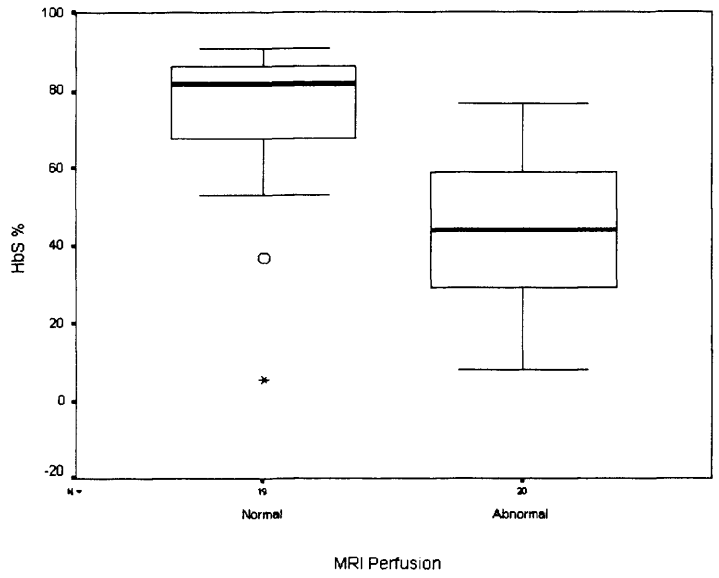


Figure 4.24. Relationship between abnormal Perfusion MRI and HbS% values (p=0.06).

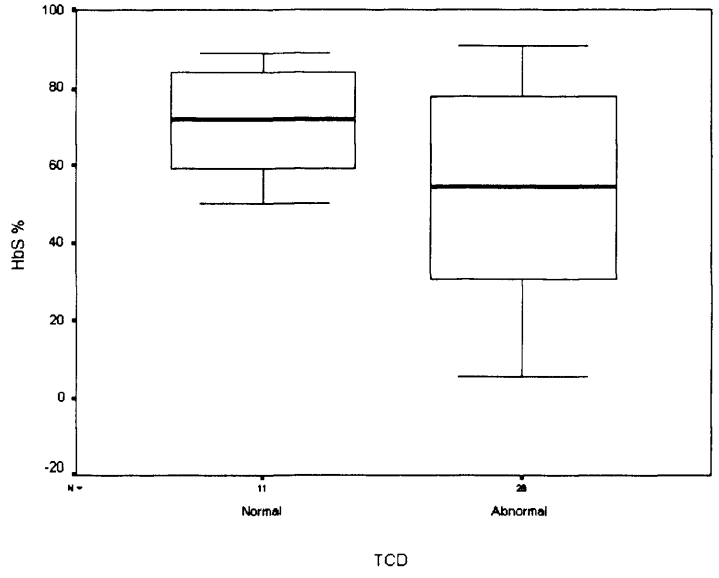


Figure 4.25. Relationship between abnormal TCD and HbS% values (p=0.002).

4.4.3.3. White Cell Count

White Cell Count (WCC): White cell count data were collected from 61 patients. Median WCC was 12.5 x 10^9/L cells, with a range from 2.3 to 51.50 x 10^9/L cells.

Table 4.12 shows the WCC by age compared with the normal values (Nelson et al 1996). There was no significant difference in the means of WCC by age group (p=0.98, one-way ANOVA)

Patients Age	**WCC x 10^9/L Mean**	**WCC x 10^9/L Range**	**Normal Values WCC x 10^9/L (not SCD)**
6-18 months (n=2)	14	9.6 -18.5	*6 - 17.5*
2-5 years (n=1)	13.5	13.5	*1-3 y: 6 -17.5* *4-7 y: 5.5-15.5*
6-12 years (n=27)	12.9	2.3 – 51.5	*4-7 y: 5.5-15.5* *8-13y: 4.5-13.5*
13-17 years (n=18)	12.1	3.7 – 25.3	*14-28y: 4.5- 11*
18-28 years (n=13)	11.8	7.2 – 15.5	*14-28y: 4.5- 11*

Table 4.12. White cell count in relation to age groups in sickle cell patients.

In relation to CNS events at presentation, the WCC mean of each symptom is shown in table 4.13. There was a significant difference between CNS symptoms at onset and WCC (p=0.009, ANOVA). Post-hoc analysis showed that patients with coma had significantly higher WCC count in relation to those with learning difficulty (p=0.03); headaches (p=0.002); seizures (p=0.01); posterior territory TIA (p=0.004); anterior territory TIA (p=0.003) and stroke (p=0.001; figure 4.26).

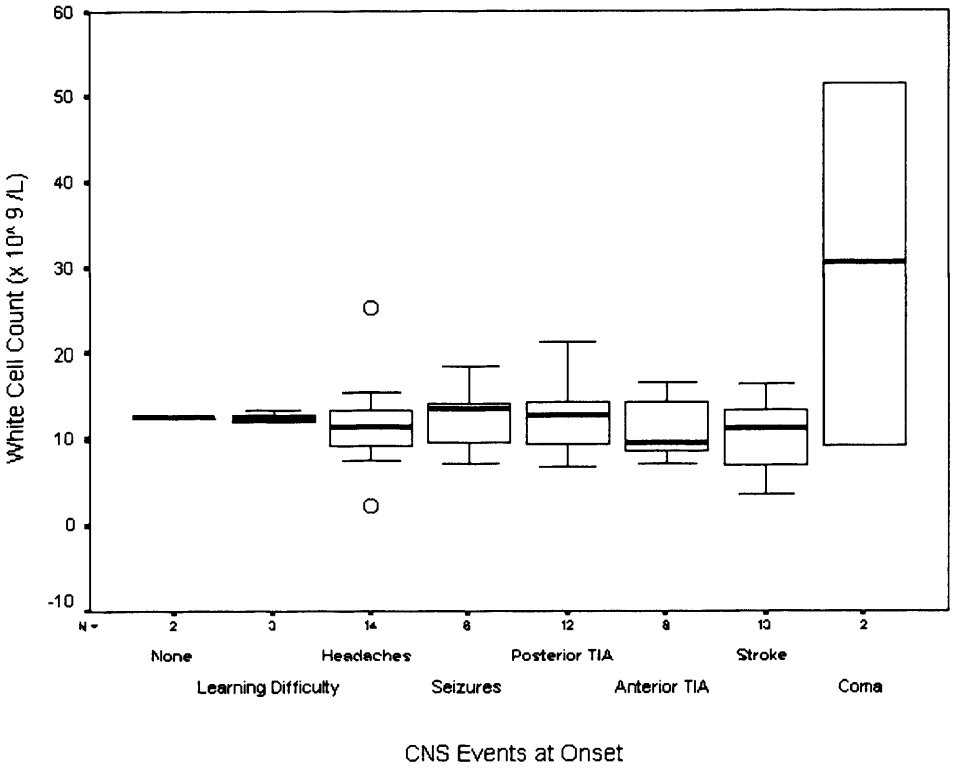


Figure 4.26. White cell count in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

CNS Events (n =Patients)	WCC x 10^9/L Mean	WCC x 10^9/L Range
No Symptoms (n=2)	12.5	12.5- 12.5
Learning Difficulty (n=3)	12.6	12- 13.5
Headaches (n=14)	11.8	2.3 – 25.3
Seizures (n=6)	12.8	7.2 – 18.5
Posterior TIA (n=12)	12.5	6.7 – 21.4
Anterior TIA (n=8)	11.2	7.1 – 16.6
Stroke (n=13)	10.4	3.7- 16.5
Coma (n=2)	30.5*	9.4 -51.5

Table 4.13. White cell count in relation to central nervous system events at presentation. TIA= transient ischaemic attack. *p=0.009.

The means of the WCC in relation to each recurrent neurological symptom are shown in table 4.14. There was a significant difference between recurrent neurological symptoms and WCC ($p<0.0001$; ANOVA. The patient who had recurrent coma had significantly higher WCC than those patients with other recurrent symptoms. Post-hoc analysis was not performed because of the small numbers (figure 4.27).

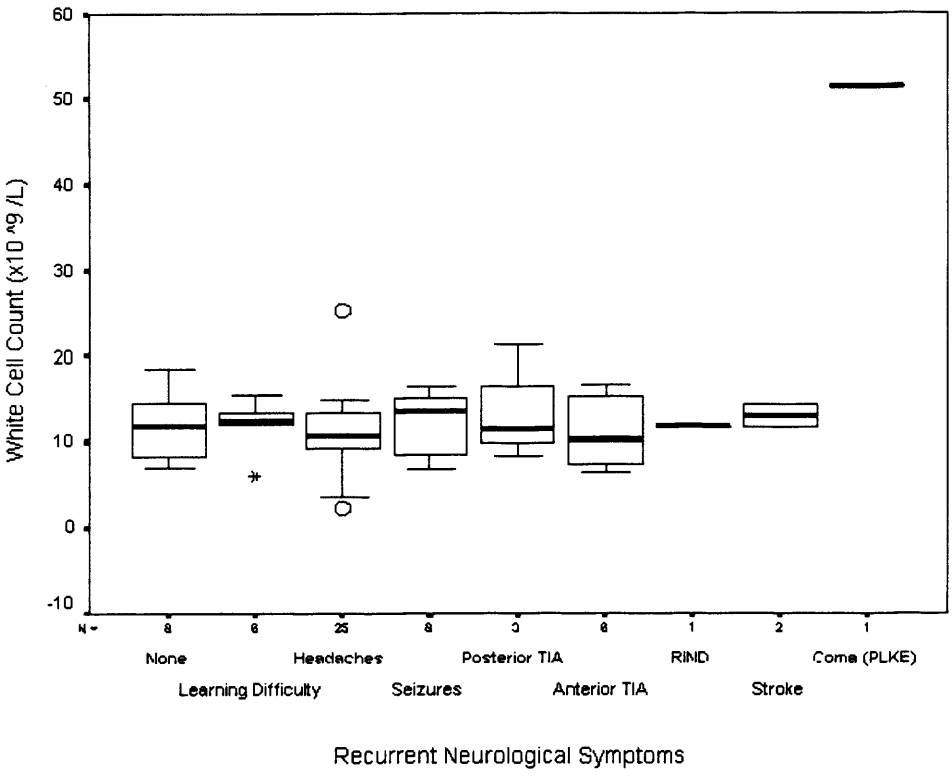


Figure 4.27. White Cell count in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Recurrent Symptom (n =Patients)	WCC x 10^9/L Mean	WCC x 10^9/L Range
No Symptoms (n=8)	11.9	6.9 - 18.5
Learning Difficulty (n=6)	11.9	5.9 – 15.4
Headaches (n=25)	11.3	2.3 – 25.3
Seizures (n=8)	12.1	6.7 – 16.5
Posterior TIA (n=3)	13.7	8.4 – 21.4
Anterior TIA (n=6)	10.9	6.3 – 16.6
RIND (n=1)	11.7	11.7
Stroke (n=2)	13.1	11.7 -14.4
Coma (PLKE) (n=1)	51.5*	51.5

Table 4.14. White Cell count in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.*$p<0.0001$

There were no significant associations between WCC and MRI (p=0.7, logistic regression); MRA (p=0.4); TCD (p=0.4); and perfusion MRI (p=0.8). Table 4.15

shows the means of WCC and reference range in relation to normal or abnormal investigations in the sickle cell patients.

Investigations (n=pats)	Normal Investigation WCC x 10^9/L Mean (Range)	Abnormal Investigation WCC x 10^9/L Mean (Range)	P value
MRI (n=60)	12 (2.3 -25.3)	12.8 (3.7 – 51.5)	p=0.7
MRA (n=59)	13.1 (2.3 – 51.5)	11.6 (3.7 – 18.5)	p=0.4
TCD (n=59)	11.3 (2.3- 18.5)	12.9 (3.7 – 51.5)	p=0.4
Perfusion MRI (n=60)	12.1 (2.3 – 25.3)	12.6 (3.7 – 51.5)	p=0.8

Table 4.15. White cell count in relation to whether MR and TCD studies are normal or abnormal.

4.4.3.4. Platelets

Platelets: Platelet count data were collected from 60 patients. Median platelet count was 372 x 10^9/L cells, with a range from 54 to 734 x 10^9/L cells (normal 150-400 x 10^9/L cells).

The mean and range for platelets in relation to the patients' CNS events at presentation and recurrent neurological symptoms are shown in tables 4.16 and 4.17. There were no significant differences between the means for platelet count of each group of CNS events (p=0.9, one-way ANOVA; figure 4.28.), or between platelet count and type of recurrent neurological symptoms (p=0.7, ANOVA; figure 4.29).

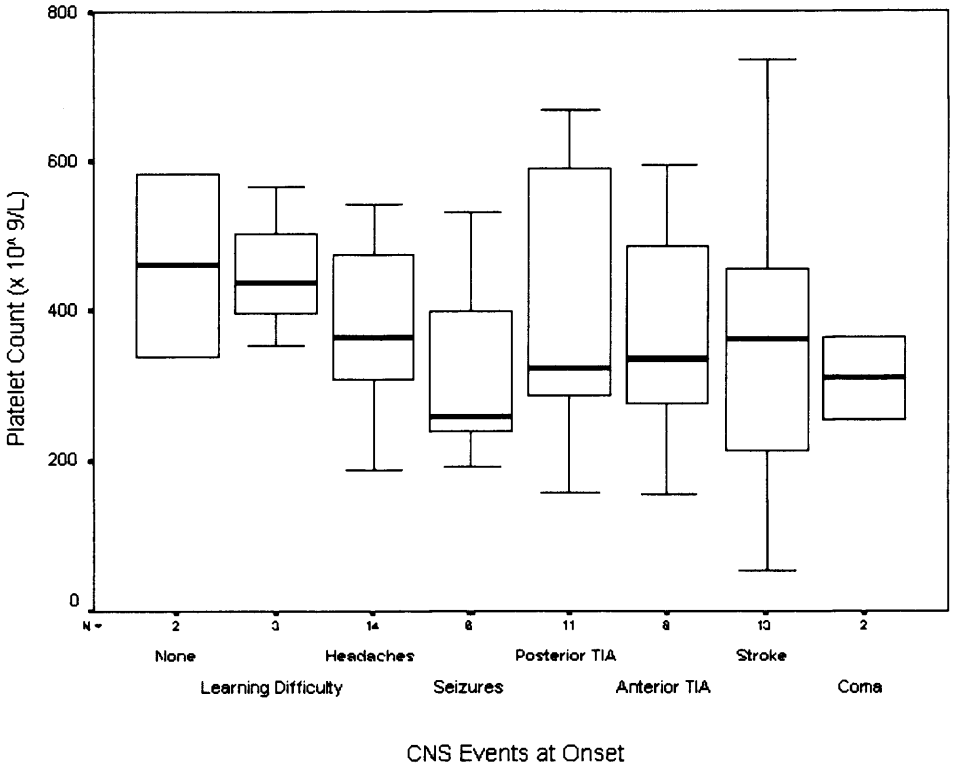


Figure 4.28. Platelet count in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

CNS Events (n =Patients)	Platelets x 10^9/L Mean	Platelets x 10^9/L Range
No Symptoms (n=2)	460	337 - 583
Learning Difficulty (n=3)	452	353 - 566
Headaches (n=14)	377	186 - 542
Seizures (n=6)	313	192 - 532
Posterior TIA (n=11)	421	157 - 669
Anterior TIA (n=8)	368	155 - 594
Stroke (n=13)	341	54- 734
Coma (n=2)	310	254 - 366

Table 4.16. Platelet count in relation to central nervous system events at presentation. TIA= transient ischaemic attack.

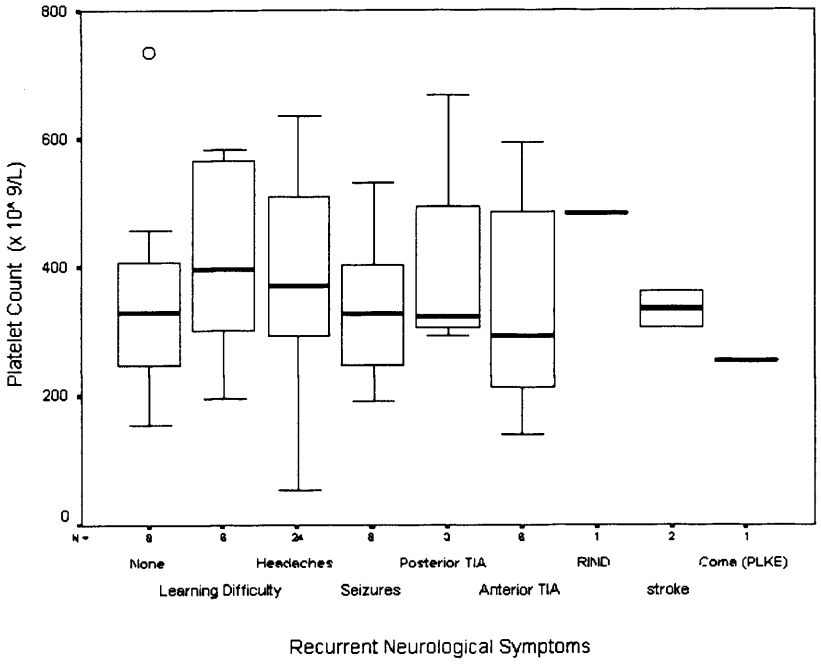


Figure 4.29. Platelet count in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Recurrent Symptom (n = Patients)	Platelets x 10^9/L Mean	Platelets x 10^9/L Range
No Symptoms(n=8)	358	155 - 734
Learning Difficulty(n=6)	406	195 - 583
Headaches (n=24)	391	54 - 636
Seizures (n=8)	335	192 - 532
Posterior TIA (n=3)	428	292 - 669
Anterior TIA (n=6)	337	140 - 594
RIND (n=1)	484	484
Stroke (n=2)	334	306 - 362
Coma (PLKE) (n=1)	254	254

Table 4.17. Platelet count in relation to recurrent neurological symptoms. TIA= transient ischaemic attack; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

There were no significant associations between platelet count and the presence of cerebral infarction on MRI (p=0.4; logistic regression); abnormal MRA (p=0.6); abnormal TCD (p=0.2); and abnormal perfusion MRI (p=0.8; table 4.18).

Investigations (n=patients)	Normal Investigation Platelet x 10^9/L Mean (Range)	Abnormal Investigation Platelet x 10^9/L Mean (Range)	P Value
MRI (n=59)	379 (155 – 669)	371 (54 – 734)	p=0.4
MRA (n=58)	365 (157 – 593)	386 (54 – 734)	p=0.6
TCD (n=58)	392 (157 – 669)	349 (54 – 734)	p=0.2
Perfusion MRI (n=59)	379 (155 – 669)	371 (54 – 734)	p= 0.8

Table 4.18. Platelet count in relation to whether MR and TCD studies are normal or abrnomal.

4.4.4. Awake Oxygen Saturation

Awake-oxygen saturation (SpO_2) was continuously recorded with a pulse oximeter for three minutes (see Methods, chapter 3) in 57 of 70 patients with sickle cell disease, just before or after the MR studies, and in 14 controls.

In sickle cell patients, the mean awake- SpO_2 was 95.3 %, range 85 to 99.9%. As described in chapter 2, the cut-off for hypoxaemia was $SpO_2 < 92\%$; 7 of 50 patients had awake-SpO_2 less than 92% (3 patients had SpO_2 between 90 to 92% and 4 had awake-SpO_2 between 85-90%). None of the patients had awake SpO_2 less than 80%.

For the controls without sickle cell disease, the mean SpO_2 was 97.8%, with a range from 95.4% to 99%. Sickle cell patients had significantly lower awake-SpO_2 values than controls (p=0.004, one way- ANOVA; figure 4.30).

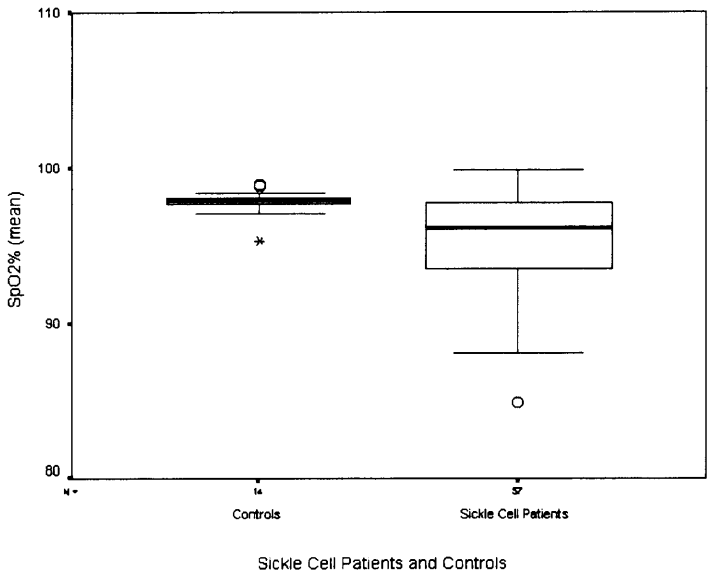


Figure 4.30. Comparison of mean awake-SpO_2 values between controls and sickle cell patients (p=0.004, one way-ANOVA).

4.4.4.1. Awake Oxygen Saturation in Relation to Sickle Cell Patients and their Central Nervous System Events at Presentation

In this series of patients with SCD, awake oxygen saturations were not significantly associated with the presence or absence of central nervous system (CNS) events at presentation (p=0.7, logistic regression) or with the presence or absence of neurological symptoms such as stroke, transient ischaemic attack (TIA) and seizures (p=0.2, logistic regression).

The mean and range of awake-SpO_2 values among the different neurological symptoms at presentation were the following (figure 4.31): asymptomatic patients 94.5% (90.9 – 98.1%); learning difficulty 94.3% (90.5-98%); headaches 94.9% (88.1- 97.8%); seizures 96.9% (92.8- 98.7%); posterior territory TIA 94.8% (88.9- 97.8%); anterior territory TIA 96.5% (92.6-97.9%); stroke 95.7% (85-99.9%); and coma 94.9% (89.9-98.3%). There were no significant differences when comparing the means of awake-SpO_2 values among groups (p=0.9, one way-ANOVA) and post-hoc analysis (p=0.9 Tukey's test). In addition, 1 of the 2 sickle cell patients with no symptoms, 1/2 with learning difficulty,

1/14 with headaches, 1/13 with posterior territory TIA (1/13), 2/13 with stroke and 1/3 with coma had awake-SpO_2 values less than 92%.

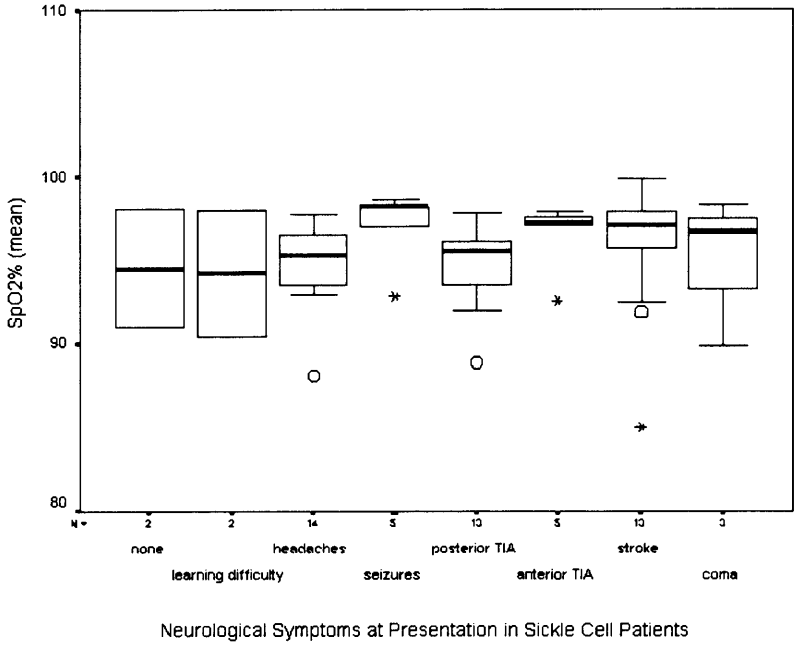


Figure 4.31. Comparison of awake-SpO_2 values and central nervous system events at presentation in sickle cell patients.

4.4.4.2. Awake Oxygen Saturation in Relation to Recurrent Neurological Symptoms in Sickle Cell Patients

There was a trend for a significant association between mean awake-SpO_2 values and the presence or absence of recurrent neurological symptoms in this series of sickle cell patients (p=0.1, logistic regression; figure 4.32), but there was no significant association with the presence or absence of neurological symptoms more associated with a vasculopathy/ischaemia cause (stroke, reversible ischaemic neurological deficit [RIND], TIAs or seizures, p=0.7, logistic regression).

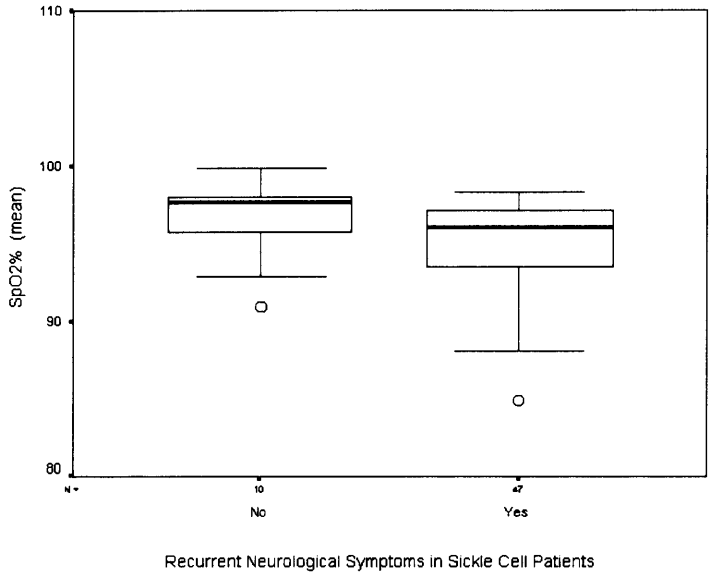


Figure 4.32. Comparison of mean awake-SpO_2 values between sickle cell patients who had recurrent neurological symptoms or remained symptom-free (p=0.1).

The mean and range of awake-SpO_2 values among the different recurrent neurological symptoms were the following (figure 4.33): asymptomatic patients 96.7% (90.9-99.9%); learning difficulty 94% (90.5-98%); headaches 95% (88.1-98.4%); seizures 96.4% (91.9-98.2%); posterior territory TIA 94.4% (89.9-97.3%); anterior territory TIA 96.6% (92.5-97.9%); RIND 96.1 % (96.1%); stroke 88.7% (85-92.5%); and coma (posterior leukoencephalopathy [PLKE]) 98.3% (98.3%). The comparison of the means of awake-SpO_2 values among groups was significant (p=0.04, one way-ANOVA).

Post-hoc analysis of the SpO_2 means among groups (excluding RIND and coma as they were single cases) showed significant differences, with lower mean awake-SpO_2 values in the stroke group in relation to asymptomatic patients (p=0.01), headaches (p=0.05), seizures (p=0.02), and anterior territory TIA (p= 0.02). There were no significant differences in mean awake-SpO_2 values among the other groups of recurrent neurological symptoms (p=0.8, Tukey's test). However, in addition to the patient who had recurrent stroke (1 out of 2), there were 1/10 patients with no recurrent symptoms, 1/3 with recurrent learning difficulty, 2/25 with headaches, 1/6 with seizures and 1/4 with posterior territory TIA who had mean awake-SpO_2 values less than 92%.

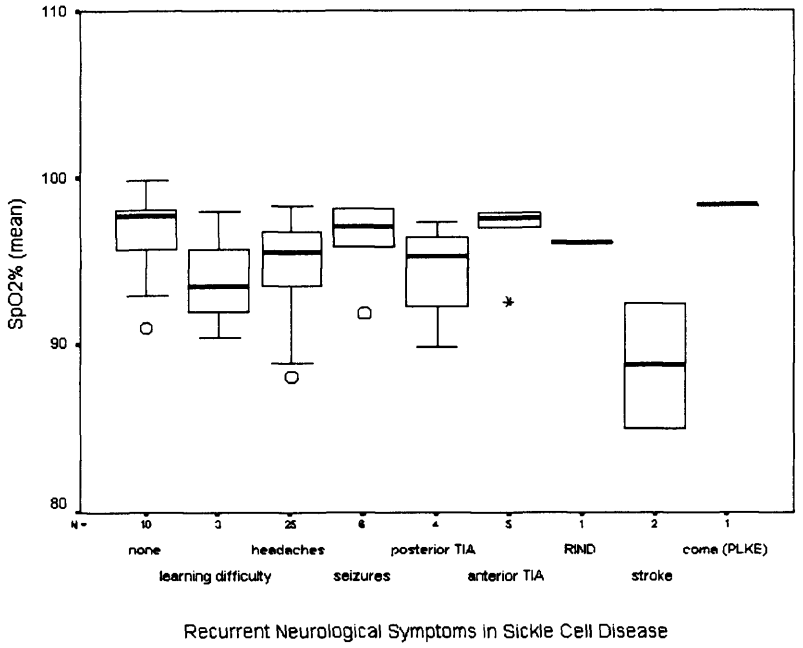


Figure 4.33. Comparison of mean awake-SpO_2 values among the recurrent neurological symptoms in sickle cell patients (p=0.04, one way- ANOVA).

4.5. Results II: Neuroimaging and Transcranial Doppler Ultrasound

4.5.1. Magnetic Resonance Imaging

Seventy patients with sickle cell disease underwent structural MRI brain.

4.5.1.1. Magnetic Resonance Imaging and Central Nervous System Events at Presentation

Forty-two of the 70 patients with sickle cell disease had normal structural MRI scan. Three out of 42 patients with normal MRI did not have neurological symptoms, and 39 patients had a main CNS event at presentation: 2 had coma, 15 had transient ischaemic attacks -TIA- (5 patients had anterior territory TIA and 10 posterior territory TIA), 6 had seizures, 13 had headaches, and 4 patients had learning difficulties. One patient had seizures and generalised cerebral atrophy bilaterally; however, for the purposes of data analysis, the MRI was classified as normal in tables 4.19 to 4.25 because there was no

focal abnormality such as infarction (therefore including this patient, the total number of patients with normal MRI is 43 in the tables 4.19 to 4.25).

Twenty-eight of the 70 patients had abnormal structural MRI: 16 had overt infarction (manifest clinically as stroke characterised by hemiparesis), 1 had cerebral atrophy (as the only MRI abnormality and without infarction) and 11 patients had covert (silent) infarction on MRI (defined as an area of increased signal intensity on T2-weighted MRI without a history of a neurological event lasting more than 24 hours [Moran et al 1998], table 4.19).

Of 16 patients with sickle cell disease and overt infarction, 11 had bilateral and 5 had unilateral cerebral infarction. The main neurological symptom at presentation was stroke (table 4.19); 2 of the 15 patients had stroke and coma.

Of the 11 patients whose scans showed covert (silent) infarcts, 8 were bilateral; the main neurological symptoms in this group were TIAs in 6 (4 patients had anterior territory TIA and 2 had posterior territory TIA); 1 patient had headaches; and 1 patient had no neurological symptoms but he had history of severe snoring and upper airway obstruction (OSA - obstructive sleep apnoea). Three patients had unilateral covert infarcts; 2 of these patients presented with anterior territory TIA and one with headaches.

Cerebral atrophy was diagnosed in 14 patients at the time of their MRI study. Eleven of 14 patients had stroke (8 had bilateral infarcts and 3 patients had unilateral infarcts); 1 patient had stroke and coma (bilateral infarcts); 1 patient had anterior territory TIA and bilateral covert infarcts. One patient had seizures and generalised cerebral atrophy bilaterally; however, the MRI was classified as normal in the tables 4.19 to 4.25 because there was no focal abnormality such as infarction (as mentioned above).

Patients (n)	Normal MRI	Overt Infarct Unilateral	Overt Infarct Bilateral	Covert Infarct Unilateral	Covert Infarct Bilateral	Total
No Symptoms	2				1	3
Learning Difficulty	4					4
Headaches	13			1	1	15
Seizures	7 [¤ 1]					7
Post. TIA	10				4	14
Ant. TIA	5			2	2 [¤ 1]	9
Stroke		5 [¤ 3]	9 [¤ 8]			14
Coma	2		2 [¤ 1]			4
Total	43	5	11	3	8	70

Table 4.19. Relationship between main neurological symptoms and T2-weighted MRI findings in 70 patients with sickle cell disease.
[¤ number of patients] = **Indicates** the number of patients with **cerebral atrophy** (e.g. 3 out of 5 patients with unilateral overt infarct had cerebral atrophy); for the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction (e.g. 1 out of 7 patients).

The grade of severity of the central nervous system events at presentation in this series of sickle cell patients was significantly associated with the presence or absence of infarcts ($p<0.0001$, Mann-Whitney test), with unilateral infarcts ($p=0.03$, Fisher's test) and bilateral infarcts ($p<0.0001$, Fisher's exact test).

Covert (silent) infarction was strongly associated with bilateral location in this series ($p<0.0001$ and $p=0.03$ respectively, Fisher's exact test). Covert infarction was significantly associated with TIA and anterior territory TIA as CNS events at presentation ($p=0.004$ and $p=0.03$, respectively, Fisher's exact test), but there were no associations with posterior territory TIA ($p=0.2$), seizures ($p=0.6$), headaches ($p=1$) or learning difficulty ($p=1$, Fisher's exact test).

4.5.1.1.1. Central Nervous System Events at Presentation and Infarct Size

Infarct size (overt and covert) was divided into three categories: 1- small (diameter < 1cm), 2- medium (1-5cm), 3-large (>5cm) (Watkins et al 1998). Thirteen patients had small infarcts; 8 patients had medium size infarcts; and 6 patients had large infarcts. The severity of central nervous system events at presentation was significantly associated with increasing infarct size ($p<0.0001$, Spearman's test, correlation

coefficient [CC] =0.6). Figure 4.34 and table 4.20 show the relationship between CNS events at presentation and infarct size.

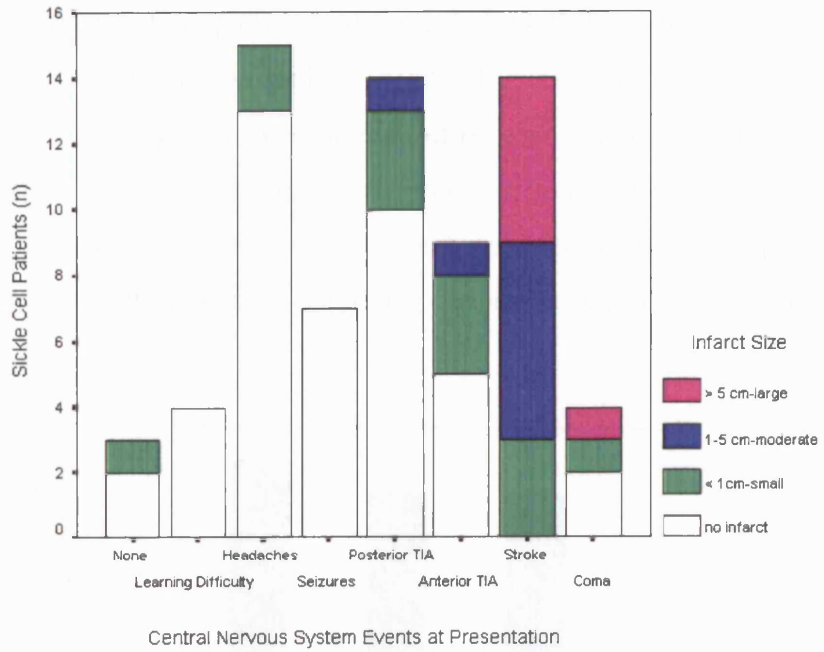


Figure 4.34. Infarct size and central nervous system events at presentation in 70 patients with SCD. TIA: transient ischaemic attack.

CNS Events (n = Patients)	Normal MRI	Infarct < 1cm-small	Infarct 1-5 cm-moderate	Infarct > 5 cm-Large	Total
No Symptoms	2	1			3
Learning Difficulty	4				4
Headaches	13	2			15
Seizures	7				7
Post TIA	10	3	1		14
Ant TIA	5	3	1		9
Stroke		3	6	5	14
Coma	2	1		1	4
Total	43	13	8	6	70

Table 4.20. Infarct size and central nervous system events at presentation in 70 patients with SCD. TIA: transient ischaemic attack.
For the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction.

4.5.1.1.2. Central Nervous System Events at Presentation and Infarct Number

Infarct number was divided into four categories (1, 2, 3 and 4 or more [multiple] infarcts). Four patients had one infarct. Five patients had 2 infarcts, 2 with unilateral and 3 with bilateral infarcts. One patient had three infarcts bilaterally; and 17 patients had multiple infarcts, 2 unilaterally, and 15 bilaterally. The severity of central nervous system events at presentation was significantly associated with increasing infarct number ($p<0.0001$, Spearman's test, correlation coefficient [CC]=0.5, figure 4.35 and table 4.21). In addition, there was a significant association between increased infarct number and decreased infarct size ($p<0.0001$, Spearman's test, correlation coefficient: 0.9, table 4.22).

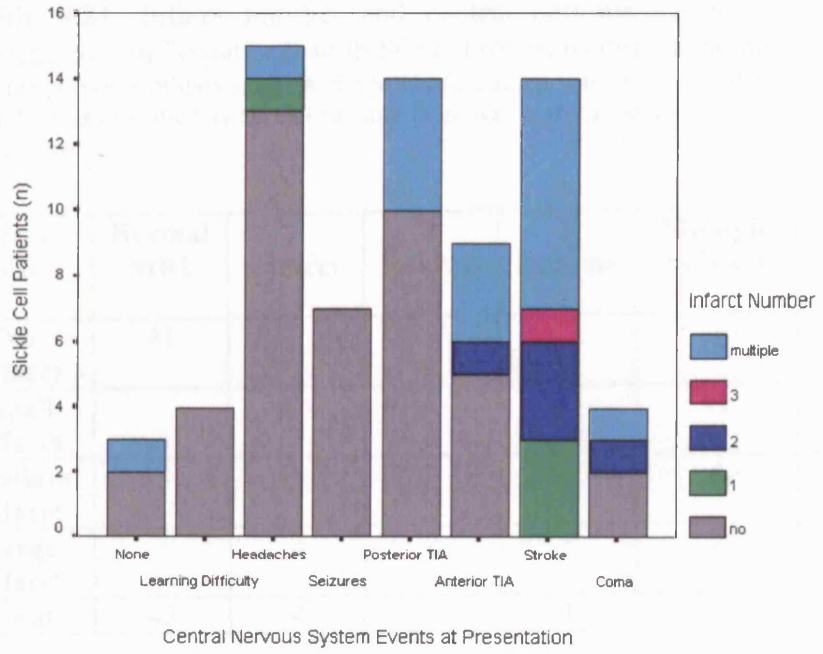


Figure 4.35. Infarct number and central nervous system events at presentation in 70 patients with SCD. TIA: transient ischaemic attack.

CNS Events (n = Patients)	Normal MRI	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	Total
No Symptoms	2				1	3
Learning Difficulty	4					4
Headaches	13	1			1	15
Seizures	7					7
Post TIA	10				4	14
Ant TIA	5		1		3	9
Stroke		3	3	1	7	14
Coma	2		1		1	4
Total	43	4	5	1	17	70

Table 4.21. Infarct number and central nervous system events at presentation in 70 patients with SCD. TIA: transient ischaemic attack.
For the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction.

Infarct Size	Normal MRI	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	Total
No Infarct	43					43
Small Infarct		1	1		11	13
Medium Infarct		1	2	1	4	8
Large Infarct		2	2		2	6
Total	43	4	5	1	17	70

Table 4.22. Relation between infarct number and infarct size in 70 patients with SCD. TIA: transient ischaemic attack. Infarct size: small =<1cm; medium=1-5cm; and large=>5cm of diameter.
For the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction.

4.5.1.2. Magnetic Resonance Imaging and Recurrent Neurological Symptoms

In relation to recurrent neurological symptoms, 7 of 42 patients with normal MRI (i.e. excluding the single patient with cerebral atrophy [see tables 4.19 to 4.25]) were asymptomatic; however, 35 patients with a normal MRI study had recurrent

neurological symptoms such as posterior territory TIA (n=3), seizures (n=6), headaches (n=22), and learning difficulties (n=5).

Twenty-eight of the 70 patients with recurrent neurological symptoms had abnormal structural MRI. Sixteen patients had had overt infarction, 1 had cerebral atrophy without infarction (included as normal in the table [a patient with seizures]) and 11 had covert (silent) infarction on MRI, table 4.23)

Of 16 patients with sickle cell disease and overt infarction, 11 had bilateral and 5 had unilateral cerebral infarction. The recurrent neurological symptoms of this group were coma (with posterior leukoencephalopathy, n=1), stroke (n=2), RIND (n=1), anterior territory TIA (n=3), seizures (n=2), headaches (n=3) and learning difficulty (n=1); three patients had no symptoms.

Eleven of the 70 patients had covert (silent) infarct. Eight patients had bilateral and 3 patients had unilateral covert infarcts; the main neurological symptoms in this group were anterior territory TIA (n=3), posterior territory TIA (n=1), headaches (n=4) and learning difficulty (n=1); two patients had no symptoms.

Cerebral atrophy on MRI was found in 12 patients with recurrent symptoms: 1 of the two patients with recurrent stroke (bilateral infarcts), 1 patient with stroke and coma (bilateral infarcts); 1 with reversible ischaemic neurological deficit - RIND - (bilateral infarcts), 3 patients with anterior territory TIA (1 with bilateral and 1 with unilateral overt infarcts; and 1 patient with covert infarcts), 3 patients with seizures (1 patient with cerebral atrophy without infarct [included in tables 4.19 to 4.25 as normal], 1 with unilateral and 1 with bilateral overt infarcts) and 1 with learning difficulty (bilateral overt infarcts). Two patients with bilateral overt infarcts and atrophy did not have recurrent symptoms (table 4.23). There was a trend for cerebral atrophy on MRI to be commoner in those with more severe recurrent neurological symptoms (p=0.1, Mann-Whitney test), with atrophy more likely in those with recurrent anterior territory TIA, RIND and stroke.

Patients (n)	Normal MRI	Overt Infarct Unilateral	Overt Infarct Bilateral	Covert Infarct Unilateral	Covert Infarct Bilateral	Total
No Symptoms	7		3 [¤ 2]	1	1	12
Learning Difficulty	5		1 [¤ 1]		1	7
Headaches	22	2 [¤ 2]	1 [¤ 1]	1	3	29
Seizures	6 [¤ 1]	1 [¤ 1]	1 [¤ 1]			8
Post. TIA	3				1	4
Ant. TIA		1 [¤ 1]	2 [¤ 1]	1	2 [¤ 1]	6
RIND			1 [¤ 1]			1
Stroke		1	1 [¤ 1]			2
Coma (PLKE)			1			1
Total	43	5	11	3	8	70

Table 4.23. Relationship between recurrent neurological symptoms and structural MRI findings in 70 patients with sickle cell disease.
[¤ number of patients] = **Indicates** the number of patients with **cerebral atrophy** (e.g. 2 out of 2 patients with unilateral overt infarct had cerebral atrophy); for the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction (e.g. 1 out of 6 patients).

The grade of severity of the recurrent neurological symptoms in this series of sickle cell patients was significantly associated with the presence or absence of infarcts (p=0.04, Mann-Whitney test). There was a trend for association between recurrent symptoms and bilateral infarcts, but no association for unilateral infarcts (p=0.1 and p=0.3, Fisher's exact test). There were trends for association between covert infarct and recurrent TIA and anterior territory TIA (p=0.06 and p=0.07, respectively, Fisher's exact test), but there were no associations with recurrent posterior territory TIA (p=0.5), seizures (p=0.3); headaches (p=1) or learning difficulty (p=1, Fisher's exact test).

4.5.1.2.1. Recurrent Neurological Symptoms and Infarct Size

Thirteen patients with recurrent neurological symptoms had small infarcts, 8 patients had medium size infarcts, and 6 patients had large infarcts. There was a trend for an association between the severity of the recurrent neurological symptoms and increasing infarct size (p=0.06, Spearman's test, correlation coefficient [CC] =0.2). Figure 4.36 and table 4.24 show the relationship between recurrent neurological symptoms and infarct size.

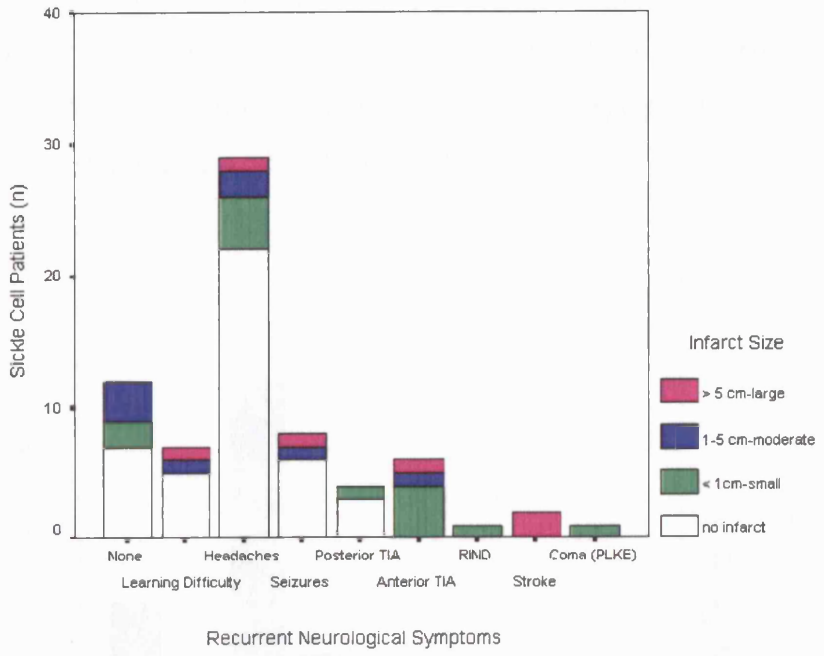


Figure 4.36. Infarct size and recurrent neurological symptoms in 70 patients with SCD. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy; RIND: reversible ischaemic neurological deficit.

Recurrent Symptom (n = Patients)	Normal MRI	Infarct < 1cm-small	Infarct 1-5 cm-moderate	Infarct > 5 cm-large	Total
No Symptoms	7	2	3		12
Learning Difficulty	5		1	1	7
Headaches	22	4	2	1	29
Seizures	6		1	1	8
Post TIA	3	1			4
Ant TIA		4	1	1	6
RIND		1			1
Stroke				2	2
Coma (PLKE)		1			1
Total	43	13	8	6	70

Table 4.24. Relationship between infarct size and recurrent neurological symptoms. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy; RIND: reversible ischaemic neurological deficit.
For the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction.

4.5.1.2.2. Recurrent Neurological Symptoms and Infarct Number

Four patients with recurrent neurological symptoms had one infarct; 5 patients had 2 infarcts; 1 patient had three infarcts; and 17 patients had multiple infarcts. There was an inverse trend for an association between the severity of recurrent neurological symptoms and decreased infarct number (p=0.07, Spearman's test, correlation coefficient [CC] = 0.2, figure 4.37 and table 4.25).

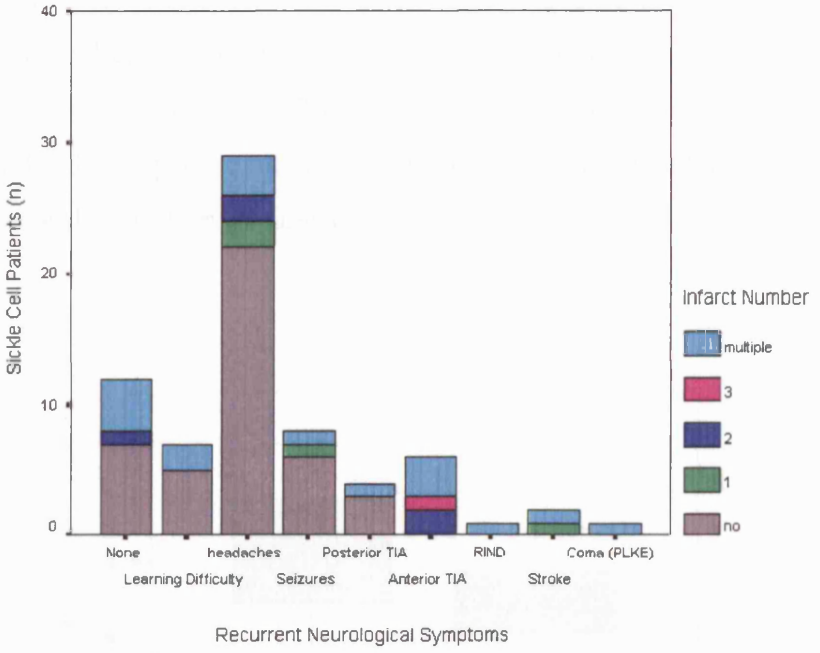


Figure 4.37. Infarct number and recurrent neurological symptoms in 70 patients with SCD. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy; RIND: reversible ischaemic neurological deficit.

Recurrent Symptom (n = Patients)	Normal MRI	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	Total
No Symptoms	7		1		4	12
Learning Difficulty	5				2	7
Headaches	22	2	2		3	29
Seizures	6	1			1	8
Post TIA	3				1	4
Ant TIA			2	1	3	6
RIND					1	1
Stroke		1			1	2
Coma(PLKE)					1	1
Total	43	4	5	1	17	70

Table 4.25. Relation between infarct number and recurrent neurological symptoms. TIA: transient ischaemic attack; PLKE: posterior leukoencephalopathy; RIND: reversible ischaemic neurological deficit. For the purposes of data analysis, the MRI of a patient with seizures and generalised atrophy was classified as normal because there was no focal abnormality such as infarction.

4.5.1.3. Magnetic Resonance Imaging and Blood Pressure Measurements

The presence of cerebral infarction on T2-weighted MRI in this group of sickle cell patients (as a whole and not taking into account age differences) was significantly associated with lower diastolic blood pressure – DBP - values (mean 53 mmHg [range 30-80 mmHg]) and mean arterial blood pressure – MAP - values (mean 72 mmHg [range 58-93 mmHg]) compared with those patients with SCD who did not have infarcts on conventional MRI and whose mean DBP was 62 mmHg - range 33-81 mmHg - with mean MAP 79 mmHg - range 55-97 mmHg - (p=0.01 and p=0.02, logistic regression, figures 4.38 and 4.39). Systolic blood pressure was not significantly associated with the presence of cerebral infarction (p=0.96).

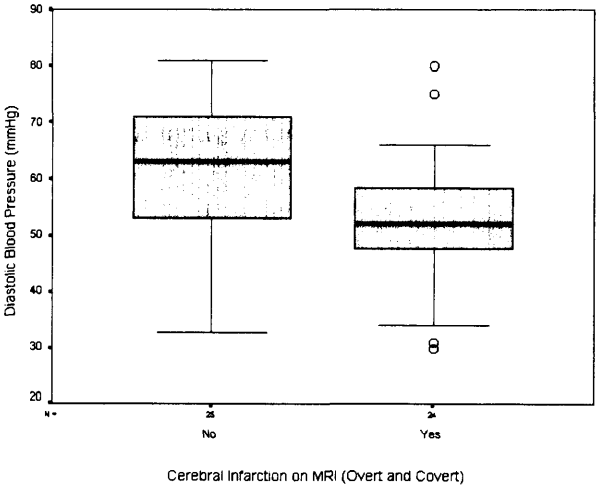


Figure 4.38 Relation between diastolic arterial blood pressure and the presence or absence of cerebral infarcts on MRI in this series of patients with SCD.

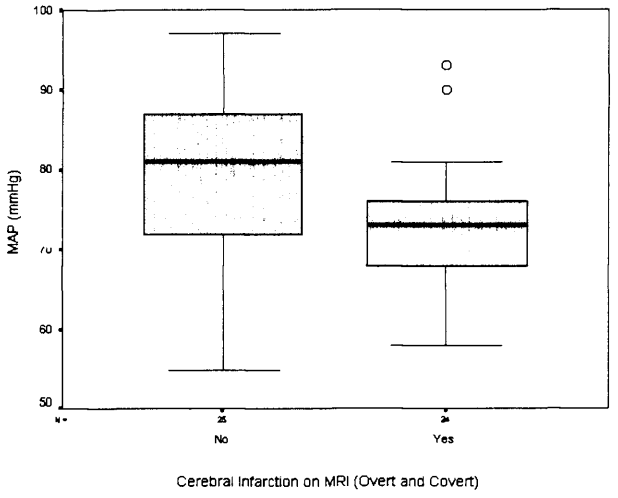


Figure 4.39. Relation between mean (MAP) arterial blood pressure and the presence or absence of cerebral infarcts on MRI in this series of patients with SCD.

4.5.1.4. Magnetic Resonance Imaging and Oxygen Saturation

Awake oxygen saturation measured by pulse oximetry (SpO_2) was not significantly associated with the presence or absence of infarcts on MRI (p=0.8, logistic regression) in this group of sickle cell patients analysed as a whole.

4.5.1.5. MRI Patterns in Relation with CNS Events at Presentation

Following the data analysis shown in this section, the predominant MRI pattern for each central nervous system event at presentation in this series of patients with SCD can be summarised as follows (from asymptomatic to most severe neurological symptom; based on infarct size [figure 4.34/ table 4.20]; and infarct number [fig. 4.35/ tab. 4.21]):

- *No symptoms:* a large proportion of patients had a normal MRI and small percentage had small, multiple infarcts;
- *Learning difficulty:* normal MRI;
- *Headaches:* mostly normal MRI and a small proportion of patient had small, multiple infarcts;
- *Seizures:* normal MRI;

- *Posterior territory TIA:* a large proportion of patients had a normal MRI , while smaller proportions had small (mainly) or moderate size, multiple infarcts;
- *Anterior TIA:* 50% of patients had normal MRI and 50% had small (mainly) or moderate size, multiple (2 or more) infarcts;
- *Stroke:* mainly moderate or large, one or multiple infarcts and a smaller proportion of patients with single small infarcts;
- *Coma:* One patient with normal MRI, and the rest with small or large, multiple infarcts.

4.5.1.6. MRI Patterns in Relation with Recurrent Neurological symptoms

Based on a single MRI study, the MRI pattern of this cross-sectional study for each recurrent neurological symptom in this series of patients with SCD can be summarised as follows (from asymptomatic to most severe neurological symptom, based on infarct size [figure 4.36/ table 4.24]; and infarct number [figure 4.37/ table 4.25]):

- *No symptoms:* two third of the patients had a normal MRI and one third had small or moderate, multiple infarcts;
- *Learning difficulty:* mostly normal MRI and a small proportion of patients had moderate or large, multiple infarcts;
- *Headaches:* mostly normal MRI and smaller proportions of patients had mainly small, moderate, or large, single or multiple infarcts;
- *Seizures:* mainly normal MRI, and smaller proportions of patients had moderate or large, single or multiple infarcts;
- *Posterior territory TIA:* a large proportion of patients had a normal MRI and smaller proportion had small, multiple infarcts;
- *Anterior TIA:* Two third of the patients had small and multiple infarcts, and one third had moderate or large, multiple infarcts;
- *Reversible ischaemic neurological deficit (RIND):* small and multiple infarcts;
- *Stroke:* large, one or multiple infarcts;
- *Coma (posterior leukoencephalopathy):* small and multiple infarcts.

4.5.2. Diffusion Weighted Imaging (DWI)

In patients with SCD and chronic infarction (n=26), DWI was as expected with high diffusion; only one of the 70 patients had an abnormal signal on DWI consisted with reduced diffusion. This patient had stroke and had neuroimaging acutely within the first week of the CNS event.

4.5.3. Magnetic Resonance Angiography

Sixty-nine out of 70 patients with SCD had a successful MRA, one patient did not tolerate this study. Thirty seven patients had a normal and 32 patients had an abnormal MRA.

4.5.3.1. MRA Turbulence (Cerebrovascular Disease) and Central Nervous System Events at Presentation

Ordinal MRA data were defined by the presence of any grade of flow turbulence seen on the scan (coded in increasing grade of severity: 0= no turbulence; 1= mild; 2= moderate; 3= severe turbulence; 4= arterial occlusion; and 5= arterial occlusion plus collaterals [moyamoya syndrome]).

Thirty-seven of the 69 patients had a normal MRA; their central nervous system events at presentation were as follows: learning difficulty (n=4), headaches (n=10), seizures (n=6), posterior territory TIA (n=8), anterior territory TIA (n=4), stroke (n=1) and coma (n=3); 1 patient had no neurological symptoms.

Of the 32 sickle cell patients with abnormal MRA, 12 patients had mild turbulence on MRA (grade 1) and they presented with headaches (n=2), seizures (n=1), posterior territory TIA (n=4), anterior territory TIA (n=1), and stroke (n=2). Two patients were asymptomatic.

Five of the 32 patients with abnormal MRA had moderate turbulence (grade 2) and these patients had headaches (n=2), anterior territory TIA (n=2) and coma (n=1).

Seven sickle cell patients had severe turbulence on MRA (grade 3) and their CNS events were the following: headaches (n=1), posterior territory TIA (n=1), anterior territory TIA (n=1) and stroke (n=4).

Three of 32 patients with abnormal MRA had arterial occlusion (grade 4) and all of them presented with stroke. Finally 5 patients with vessel occlusion and the presence of collaterals (grade 5) had posterior territory TIA (n=1) and stroke (n=4).

The severity of the CNS events at presentation was significantly associated with increasing grade of turbulence/occlusion on MRA ($p<0.0001$, Spearman's test, correlation coefficient [CC]=0.4). Figure 4.40 and table 4.26 show the association of CNS events and grades of MRA turbulence in these sickle cell patients.

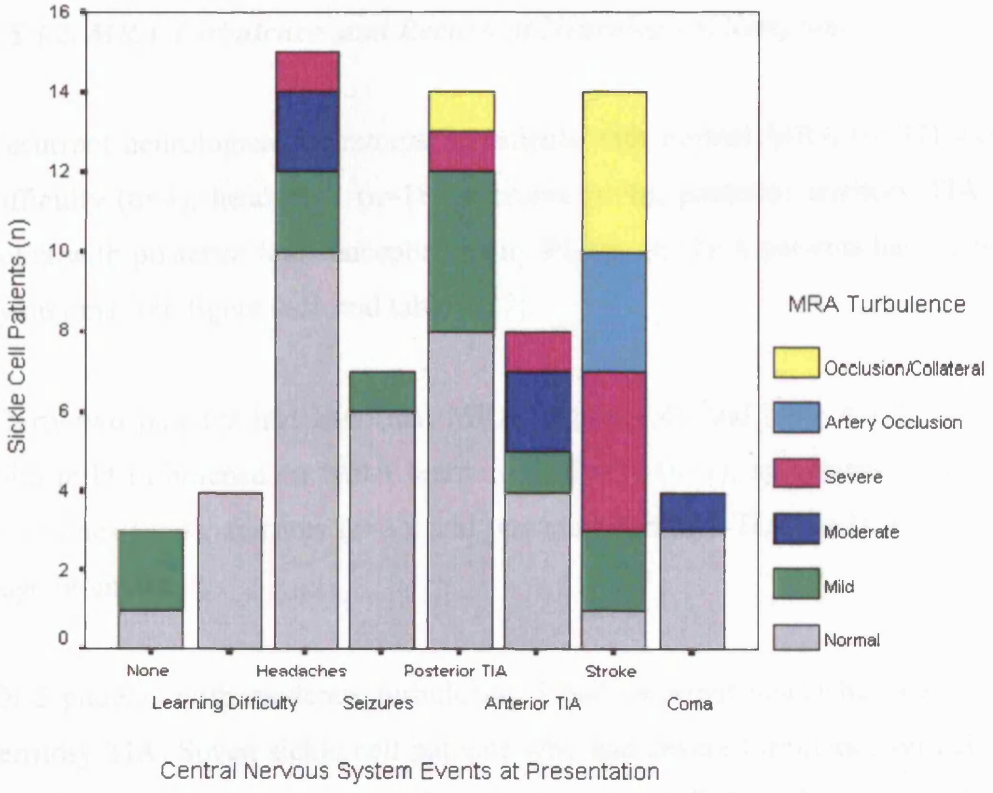


Figure 4.40. Association between central nervous system events at presentation and grades of MRA turbulence. TIA: transient ischaemic attack.

CNS Events (n = Patients)	Normal MRA	Mild Turbulence Grade 1	Moderate Turbulence Grade 2	Severe Turbulence Grade 3	Artery Occlusion Grade 4	Occlusion + Collaterals Grade 5	Total
No Symptoms	1	2					3
Learning Difficulty	4						4
Headaches	10	2	2	1			15
Seizures	6	1					7
Post TIA	8	4		1		1	14
Ant TIA	4	1	2	1			8
Stroke	1	2		4	3	4	14
Coma	3		1				4
Total	37	12	5	7	3	5	69

Table 4.26. Association between central nervous system events at presentation and grades of MRA turbulence. TIA: transient ischaemic attack.

4.5.3.2. MRA Turbulence and Recurrent Neurological Symptoms

Recurrent neurological symptoms in patients with normal MRA (n=37) were learning difficulty (n=4), headaches (n=18), seizures (n=6), posterior territory TIA (n=2), and coma with posterior leukoencephalopathy-PLKE- (n=1); 6 patients had no neurological symptoms (see figure 4.41 and table 4.27).

Thirty-two patients had abnormal MRA (figure 4.41 and table 4.27). Of 12 patients with mild turbulence on MRA learning difficulty (n=1), symptoms included recurrent headaches (n=4), seizures (n=1), and posterior territory TIA (n=2). Four patients were asymptomatic.

Of 5 patients with moderate turbulence, 3 had recurrent headaches and 2 had anterior territory TIA. Seven sickle cell patients who had severe turbulence on MRA (grade 3) continued to have headaches (n=2); anterior territory TIA (n=3), or reversible ischaemic neurological deficit (n=1). One patient remained without neurological symptoms.

Patients with vessel occlusion (n=3) had recurrent seizures (n=1), anterior TIAs (n=1) and stroke (n=1). Five patients with vessel occlusion and collaterals had learning difficulty (n=2), recurrent headaches (n=2) and stroke (n=1; figure 4.41 and table 4.27).

The severity of the recurrent neurological symptoms in these sickle cell patients was associated (but with borderline significance) with increasing grade of turbulence or pattern of vessel occlusion on MRA (p=0.056, Spearman's test, correlation coefficient [CC]=:0.2). Figure 4.41 and table 4.27 show the association between the recurrent neurological symptoms and grades of MRA turbulence in these sickle cell patients.

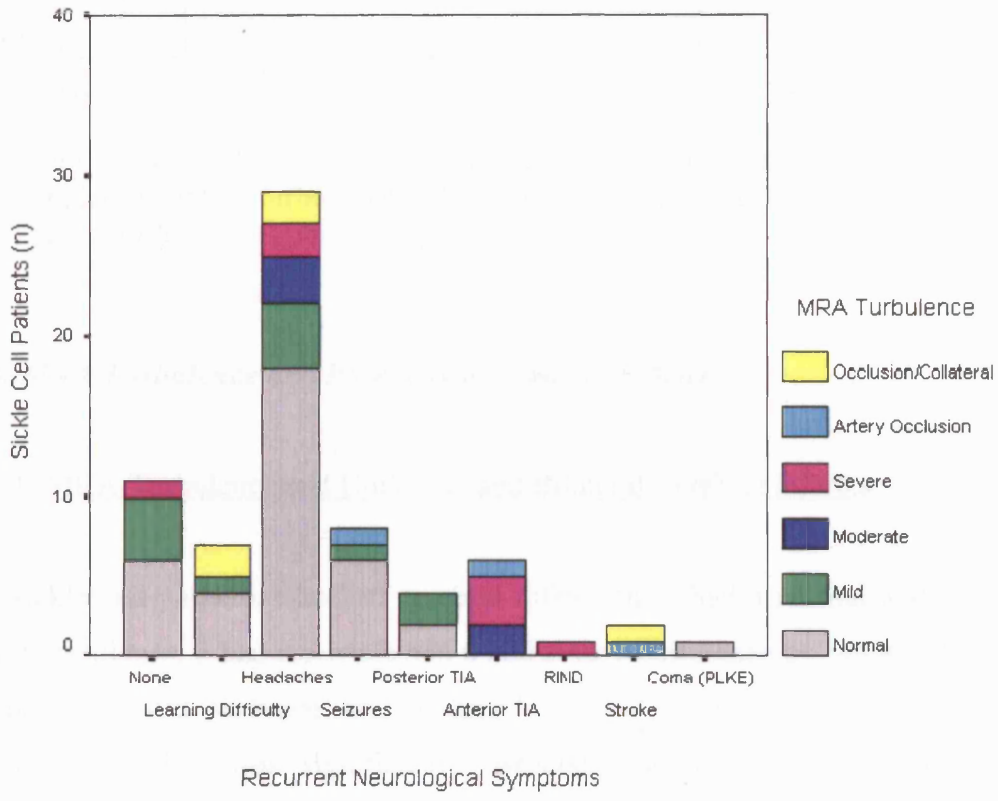


Figure 4.41. Association of recurrent neurological symptoms and grades of MRA turbulence. TIA: transient ischaemic attack, PLKE: posterior leukoencephalopathy

Recurrent Symptom (n =Patients)	Normal MRA	Mild Turbulence Grade 1	Moderate Turbulence Grade 2	Severe Turbulence Grade 3	Artery Occlusion Grade 4	Occlusion + Collateral Grade 5	Total
No Symptoms	6	4		1			11
Learning Difficulty	4	1				2	7
Headache	18	4	3	2		2	29
Seizures	6	1			1		8
Post TIA	2	2					4
Ant TIA			2	3	1		6
RIND				1			1
Stroke					1	1	2
Coma (PLKE)	1						1
Total	37	12	5	7	3	5	69

Table 4.27. Association of recurrent neurological symptoms and grades of MRA turbulence. TIA: transient ischaemic attack, PLKE: posterior leukoencephalopathy

4.5.3.3. MRA Turbulence and Parenchymal Imaging (MRI)

4.5.3.3.1. MRA Turbulence and Unilateral and Bilateral Cerebral Infarcts

Of the sickle cell patients who had cerebral infarction, 2 had a normal MRA study, 8 had mild turbulence, 3 had moderate and 6 had severe turbulence on MRA, 3 had artery occlusion and 5 had occlusion and collaterals (figure 4.42). Severity of the grade of turbulence on MRA was significantly associated with the presence of infarcts ($p<0.0001$), unilateral infarcts ($p<0.0001$) and bilateral infarcts ($p<0.0001$, Mann-Whitney test) on T2- weighted MRI.

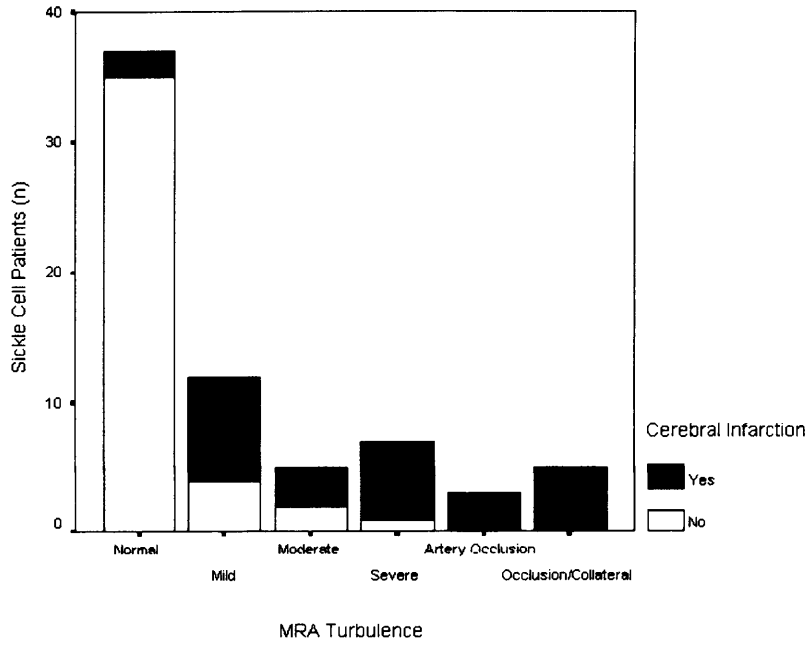


Figure 4.42. Association of cerebral infarction on MRI and grades of MRA turbulence.

Unilateral infarcts on MRI in these patients with SCD were associated in a greater proportion with artery occlusion plus collaterals; however there were also unilateral infarcts with mild and severe turbulence and artery occlusion (figure 4.43).

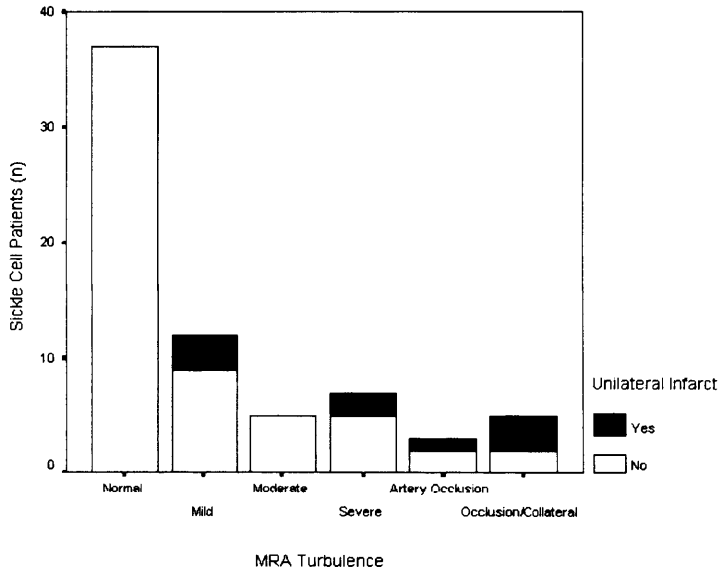


Figure 4.43. Association of unilateral cerebral infarction on MRI and grades of MRA turbulence.

Bilateral infarcts were seen mainly in those with artery occlusion and mild, moderate and severe turbulence on MRA, in addition there were a few patients with normal MRA and bilateral infarcts (figure 4.44).

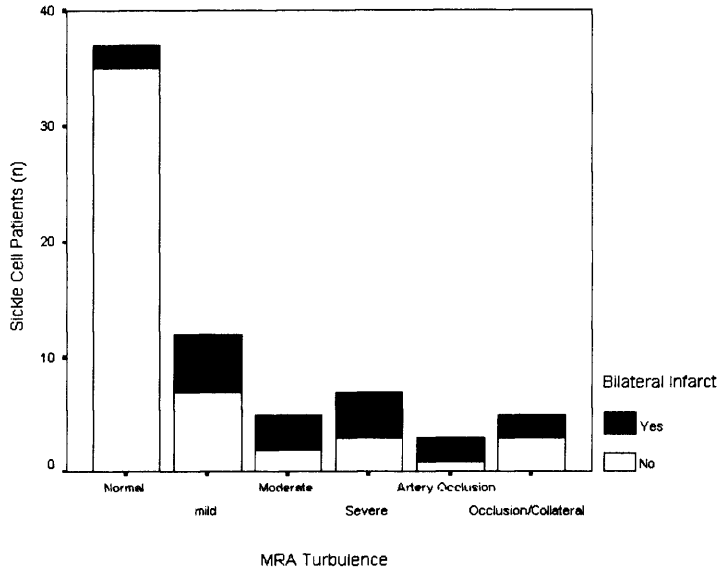


Figure 4.44. Association of bilateral cerebral infarction on MRI and grades of MRA turbulence.

4.5.3.3.2. MRA Turbulence and Covert (Silent) Infarcts

Covert infarcts on MRI were found in patients with mild (n=6), moderate (n=2) and severe turbulence on MRA (n=2), and in one patient with artery occlusion and collaterals (figure 4.45). Covert infarction was significantly associated with the grade of MRA turbulence ($p<0.0001$, Mann- Whitney test).

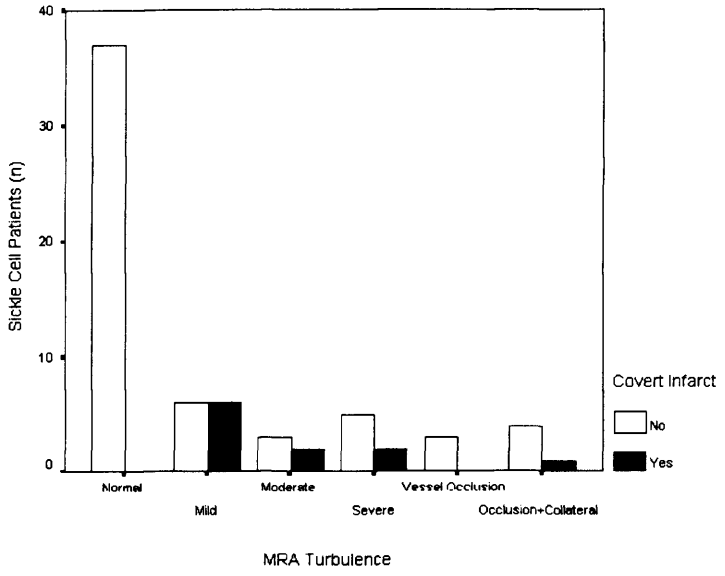


Figure 4.45. Association of bilateral covert infarction on MRI and grades of MRA turbulence.

4.5.3.3.3. MRA Turbulence and Infarct Size and Infarct Number

Worsening of MRA turbulence or vessel occlusion was significantly associated with increasing infarct size ($p<0.0001$, CC: 0.8) and infarct number ($p<0.0001$, CC: 0.7, Spearman's correlation) on MRI. Figure 4.46 and table 4.28 show the relationship between MRA abnormality and infarct size; and figure 4.47 and table 4.29 show the association between MRA grades and infarct number in this series of patients with sickle cell disease.

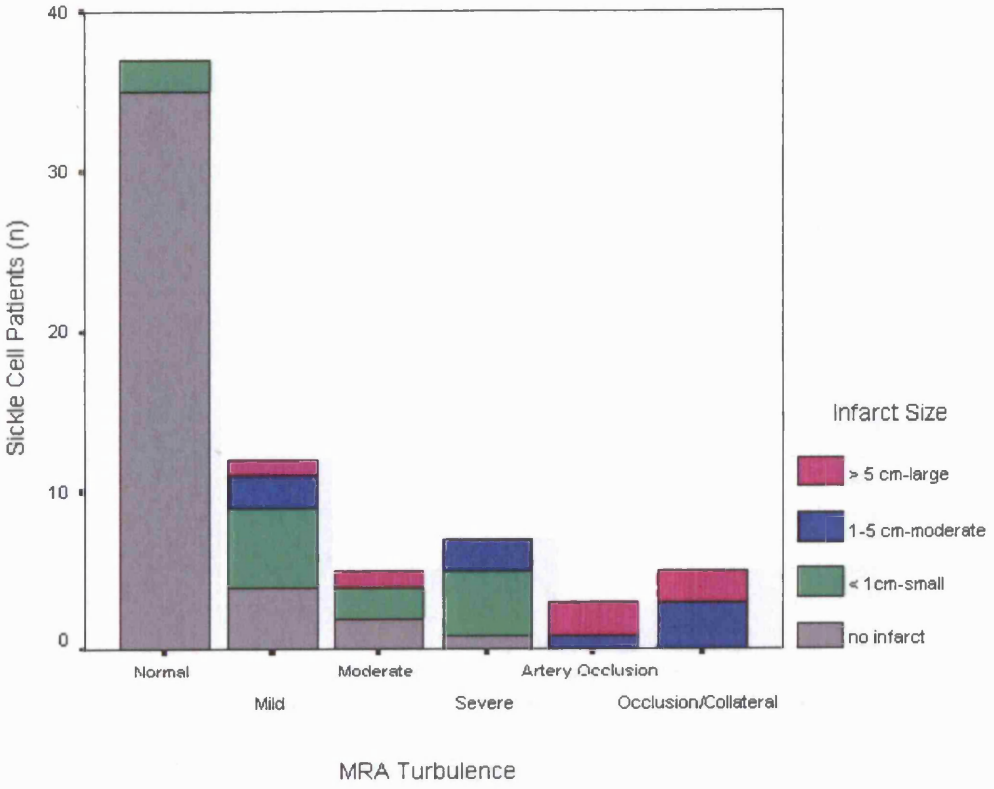


Figure 4.46. Association of infarct size and grades of MRA turbulence.

MRA (n = Patients)	Normal MRI	Infarct < 1cm-small	Infarct 1-5 cm-moderate	Infarct > 5 cm-large	Total
Normal MRA	35	2			37
Mild Turbulence	4	5	2	1	12
Moderate Turbulence	2	2		1	5
Severe Turbulence	1	4	2		7
Artery Occlusion			1	2	3
Occlusion+Collaterals			3	2	5
Total	42	13	8	6	69

Table 4.28. Relationship between infarct size and grades of MRA turbulence.

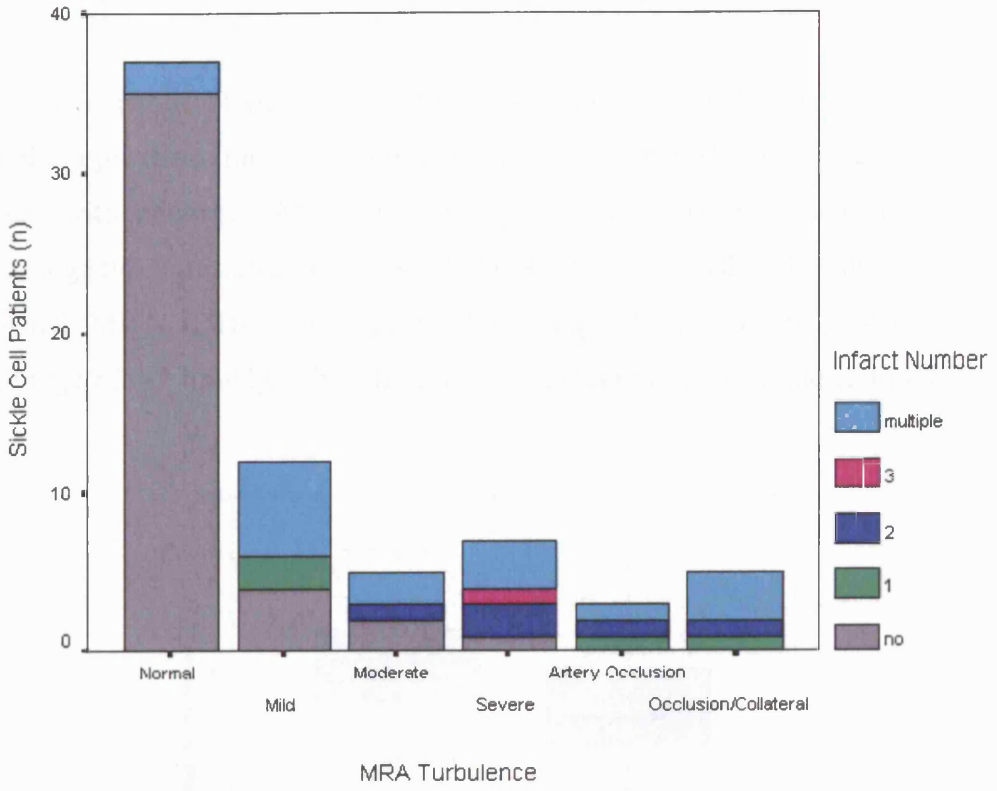


Figure 4.47. Relationship between infarct number and grades of MRA turbulence.

MRA (n = Patients)	Normal MRI	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	Total
Normal MRA	35				2	37
Mild Turbulence	4	2			6	12
Moderate Turbulence	2		1		2	5
Severe Turbulence	1		2	1	3	7
Artery Occlusion		1	1		1	3
Occlusion + Collaterals		1	1		3	5
Total	42	4	5	1	17	69

Table 4.29. Relationship between infarct number and grades of MRA turbulence.

4.5.3.4. MRA Turbulence and Blood Pressure Measurements

Lower mean diastolic blood pressure (mean 54 mmHg [range 30-80 mmHg]) and mean arterial blood pressure (mean 73 mmHg [range 55-93 mmHg]) values were significantly associated with abnormal MRA in this series of patients with SCD (p=0.047 and p=0.055, logistic regression, figures 4.48 and 4.49), compared with those patients who had normal MRA (DBP mean 62 mmHg [range 45-81 mmHg]; MAP mean 79 mmHg[range 65-97 mmHg]), but there was no association for systolic blood pressure (p=0.7).

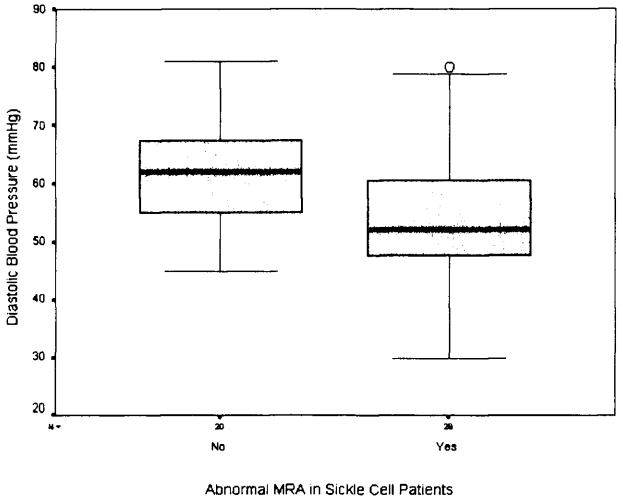
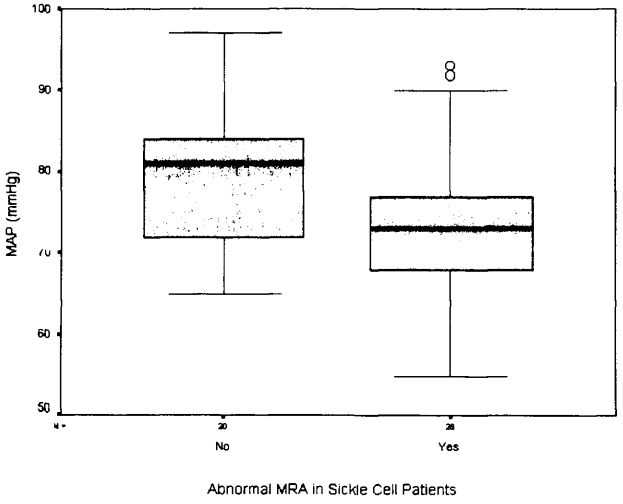


Figure 4.48. Relationship between diastolic blood pressure and MRA abnormality.



Figures 4.49. Relationship between mean arterial blood pressure measurements and MRA abnormality.

4.5.3.5. MRA Turbulence and Oxygen Saturation

There was no significant association between mean awake-SpO_2 and presence or absence of abnormal MRA in this group of patients (p= 0.5, logistic regression).

4.5.3.6. MRA Patterns in Relation to CNS Events

Following the data analysis shown in this section, the MRA pattern for each central nervous system events at presentation in this series of patients with SCD can be summarised as follow (from asymptomatic to most severe neurological symptom, figure 4.40 and table 4.26):

- *No symptoms:* Two thirds of the patients had mild turbulence on MRA and one third had normal MRA
- *Learning difficulty:* normal MRA;
- *Headaches:* a large proportion of patients had normal MRA, while smaller proportions had mild, moderate and severe turbulence;
- *Seizures:* mostly normal MRA; and few patients had mild turbulence;
- *Posterior territory TIA:* more than half of the patients had a normal MRA, one third had mild turbulence, and a small percentage had severe turbulence and artery occlusion plus collaterals ;
- *Anterior TIA:* 50% of patients had normal MRA, while smaller proportions had moderate, mild and severe turbulence;
- *Stroke:* one third of patients had severe turbulence and one third had artery occlusion plus collaterals, while smaller proportions had artery occlusion, mild turbulence and in few patients normal MRA;
- *Coma:* mainly normal MRA, with a small proportion having moderate turbulence.

4.5.3.7. MRA Patterns in Relation to Recurrent Neurological Symptoms

Based on an MRA at a single time point study, the MRA pattern for each recurrent neurological symptom in this series of patients with SCD can be summarised as follow (from asymptomatic to most severe neurological symptom, figure 4.41 and table 4.27):

- *No symptoms:* 50% of the patients had a normal MRA, while a smaller proportion had turbulence, and few patients had severe turbulence;
- *Learning difficulty:* two third of the patients had normal MRA and a small proportion of patients had occlusion plus collaterals and mild turbulence;
- *Headaches:* two third of the patients had normal MRA, while smaller proportions had mild, moderate and severe turbulence and artery occlusion plus collaterals ;
- *Seizures:* mainly normal MRA, and small proportions of patients with mild turbulence or artery occlusion plus collaterals;
- *Posterior territory TIA:* half of the patients had normal MRA, and half had mild turbulence;
- *Anterior TIA:* half of the patients had severe turbulence, while a smaller proportion of patients had moderate turbulence and few patients had artery occlusion;
- *Reversible ischaemic neurological deficit (RIND):* severe turbulence;
- *Stroke:* half of the patients had artery occlusion and half had artery occlusion plus collaterals;
- *Coma (with posterior leukoencephalopathy):* normal MRA.

4.5.4. Perfusion MRI

4.5.4.1. Perfusion Abnormality and CNS Events at Presentation

Seventy patients with SCD had a successful perfusion MRI (dynamic susceptibility contrast MRI or DSC-MRI). Thirty-one patients (44%) had normal perfusion MRI studies and 39 patients (56%) had abnormal studies. The severity of the cerebral perfusion abnormality in relation to central nervous system events at presentation is

shown in figures and tables with perfusion MRI maps of cerebral blood flow – CBF - (abnormal perfusion= decreased CBF) only, whereas perfusion MRI maps of CBF and mean transit time (MTT) of the passage of the intravenous bolus of Gadolinium (abnormal perfusion= increased MTT) were used for statistical analysis.

Thirty one patients had normal perfusion and the following main CNS events at presentation: learning difficulty (n=2), headaches (n=11), seizures (n=3), posterior territory TIA (n=8), anterior territory TIA (n=3), and coma (n=2). Two were asymptomatic.

Of the 39 patients with abnormal perfusion MRI, 5 patients (7%) had mild cerebral perfusion abnormality (mildly decreased CBF); CNS events were learning difficulty in 2, headaches in 1 and posterior territory TIA in 2 patients.

Fifteen (21%) patients had moderate perfusion abnormality (moderately decreased CBF), and their symptoms were headaches in 2, seizures in 4, posterior territory TIA in 3, anterior territory TIA in 2, stroke in 2 and coma in 1. There were no symptoms in 1 patient.

Finally, 19 sickle cell patients (27%) had severely abnormal perfusion (severely decreased CBF) and they presented with headaches (n=1), posterior territory TIA (n=1), anterior territory TIA (n=4), stroke (n=12) and coma (n=1). Figure 4.50 and table 4.30 show the relationship between perfusion abnormality on DSC-MRI and CNS events at presentation.

The severity of the central nervous system events at presentation was significantly associated with worsening perfusion MRI demonstrated for both decreased CBF and increased MTT ($p<0.0001$, coefficient correlation [CC]= 0.5, and $p<0.0001$, CC= 0.5 respectively; Spearman's test).

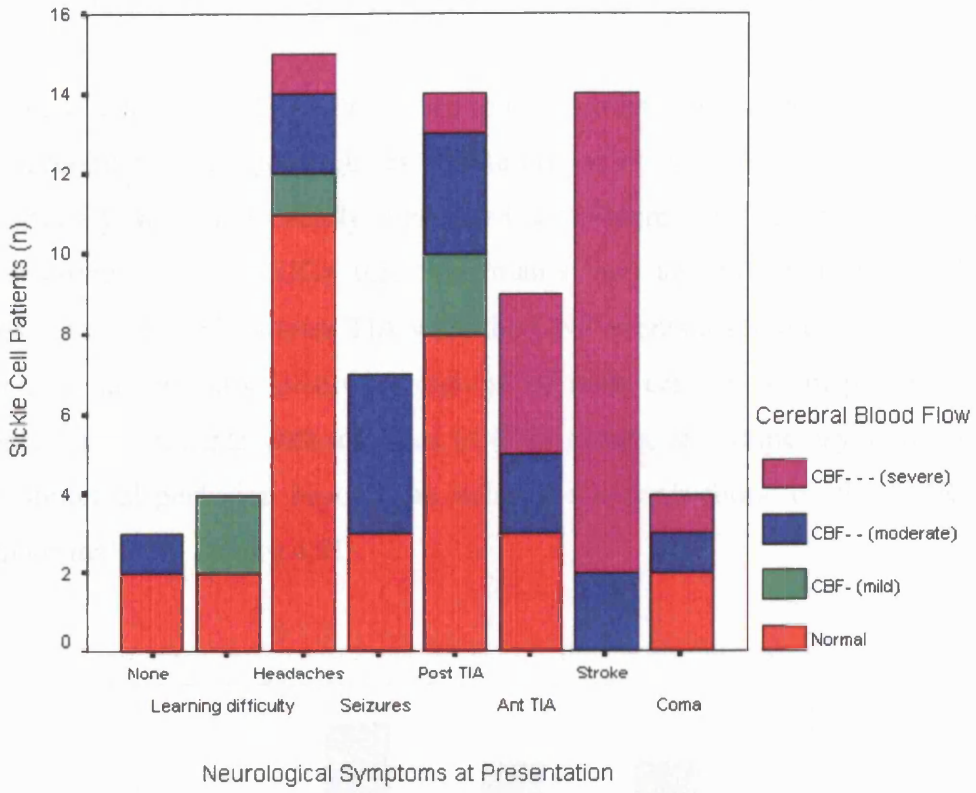


Figure 4.50. Relationship between central nervous system events at presentation and cerebral blood flow (CBF) maps on perfusion MRI. TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory.

CNS Events (n = Patients)	Normal Perf. MRI (Normal CBF)	Mild Decreased CBF (CBF-)	Moderate Decreased CBF (CBF--)	Severe Decreased CBF (CBF---)	Total
No Symptoms	2		1		3
Learning Difficulty	2	2			4
Headache	11	1	2	1	15
Seizures	3		4		7
Post TIA	8	2	3	1	14
Ant TIA	3		2	4	9
Stroke			2	12	14
Coma	2		1	1	4
Total	31	5	15	19	70

Table 4.30. Relationship between central nervous system events at presentation and cerebral blood flow (CBF) maps on perfusion MRI. TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory.

4.5.4.1.1. Extension of Perfusion Abnormality and CNS Events at Presentation

Abnormal cerebral perfusion localised to one hemisphere was found in 11 patients, and was present in both hemispheres (bilateral) in 28 patients. The extent of perfusion abnormality was significantly associated with increasing severity of CNS event at presentation (p=0.007, CC= 0.3, Spearman's test) and bilateral abnormal perfusion. Stroke, seizures and anterior TIA were the CNS events associated with more extensive perfusion abnormality involving regions of both cerebral hemispheres. However, a proportion of patients with all types of CNS events, and some asymptomatic patients, had abnormal perfusion beyond the ischaemic lesion/s found on their MRI studies or with normal MRI (figure 4.51).

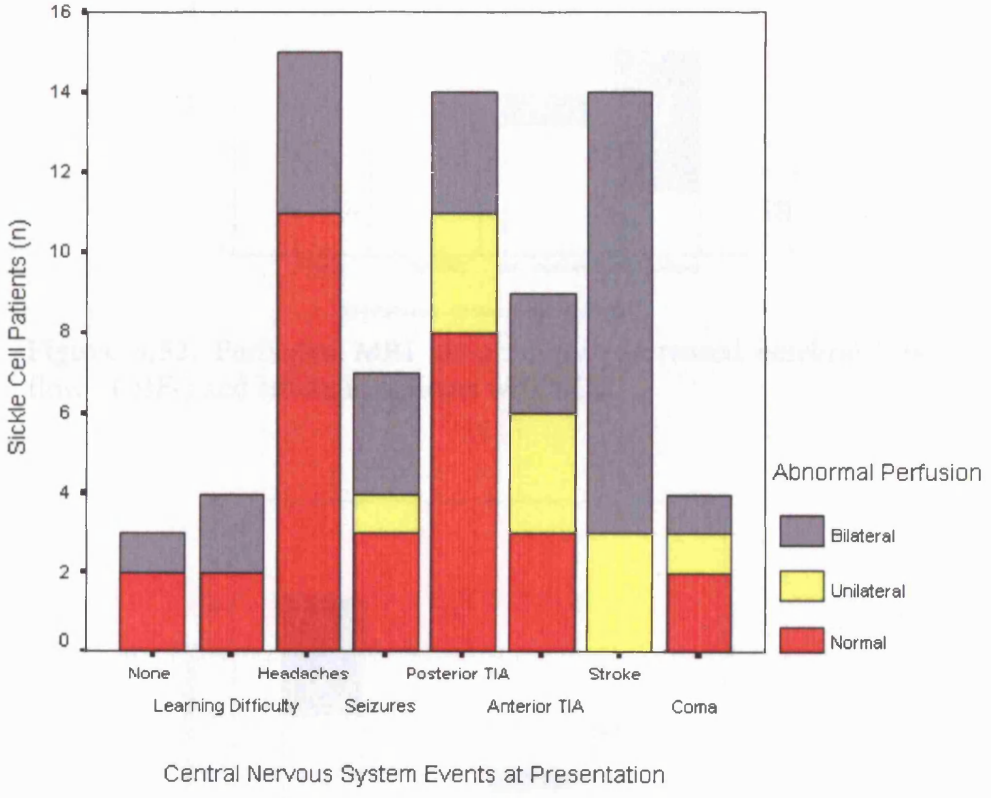


Figure 4.51. Relationship between central nervous system events at presentation and the extent of cerebral perfusion abnormality for each symptom. TIA= transient ischaemic attack. Unilateral abnormal perfusion= confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI. Bilateral abnormal perfusion= extended to regions of both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s found on MRI.

4.5.4.1.2. Perfusion Abnormality and CNS Event

The univariate analysis between each CNS event and normal/abnormal perfusion (decreased CBF) showed significant associations for stroke ($p<0.0001$, Mann-Whitney test, figure 4.52) and headaches ($p=0.008$, figure 4.53), which are the CNS events with the greatest and lowest proportion of perfusion abnormality respectively (figure 4.51). There was a trend for association with posterior territory TIA ($p=0.09$). There were no associations for anterior territory TIA ($p=0.3$), seizures ($p=0.6$) and learning difficulty ($p=0.3$). Other CNS events were not analysed because of small numbers.

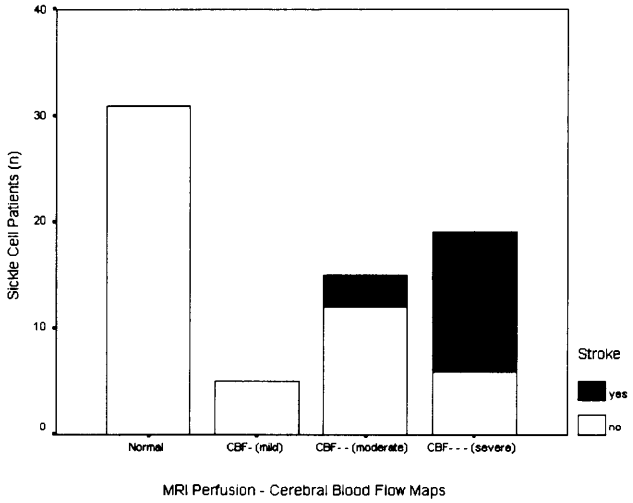


Figure 4.52. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and stroke in patients with SCD.

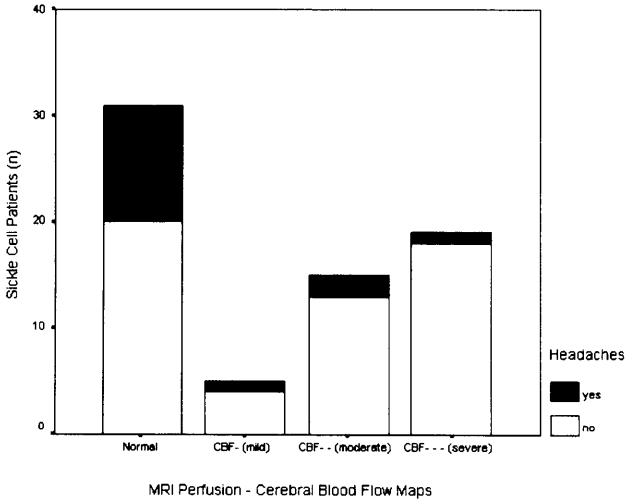


Figure 4.53. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and headaches in patients with SCD.

4.5.4.2. Perfusion Abnormality and Recurrent Neurological Symptoms

Of the 31/70 patients with normal perfusion MRI, 25 patients had recurrent neurological symptoms ongoing including learning difficulty (n=2), headaches (n=18), seizures (n=2) and posterior territory TIA (n=3).

Of the 39 patients with abnormal perfusion MRI, 5 patients with mild cerebral perfusion abnormality (mildly decreased CBF) had ongoing learning difficulty (n=2), recurrent headaches (n=1), seizures (n=1) and posterior territory TIA (n=3).

Fifteen patients with moderate perfusion abnormality (moderately decreased CBF) had recurrent symptoms such as ongoing learning difficulty in 1 patient, headaches in 6, seizures in 4, anterior territory TIA in 1 and coma with posterior leukoencephalopathy (PLKE) in 1. Two patients were asymptomatic.

Nineteen sickle cell patients with severely abnormal perfusion (severely decreased CBF) had ongoing learning difficulty (n=2), recurrent headaches (n=4), seizures (n=1), anterior territory TIA (n=5), reversible ischaemic neurological deficit (RIND, n=1) and stroke (n=2). Figure 4.54 and table 4.31 show the relationship between perfusion abnormality on DSC-MRI and recurrent neurological symptoms in this series of patients with SCD.

There was a trend for an association between the severity of the recurrent neurological symptoms and worsening perfusion MRI demonstrated by decreased CBF (p=0.1, CC= 0.2, Spearman's test) but there was no association with an increased MTT (p=0.2, [the correlation coefficient is not included because it is not significant]).

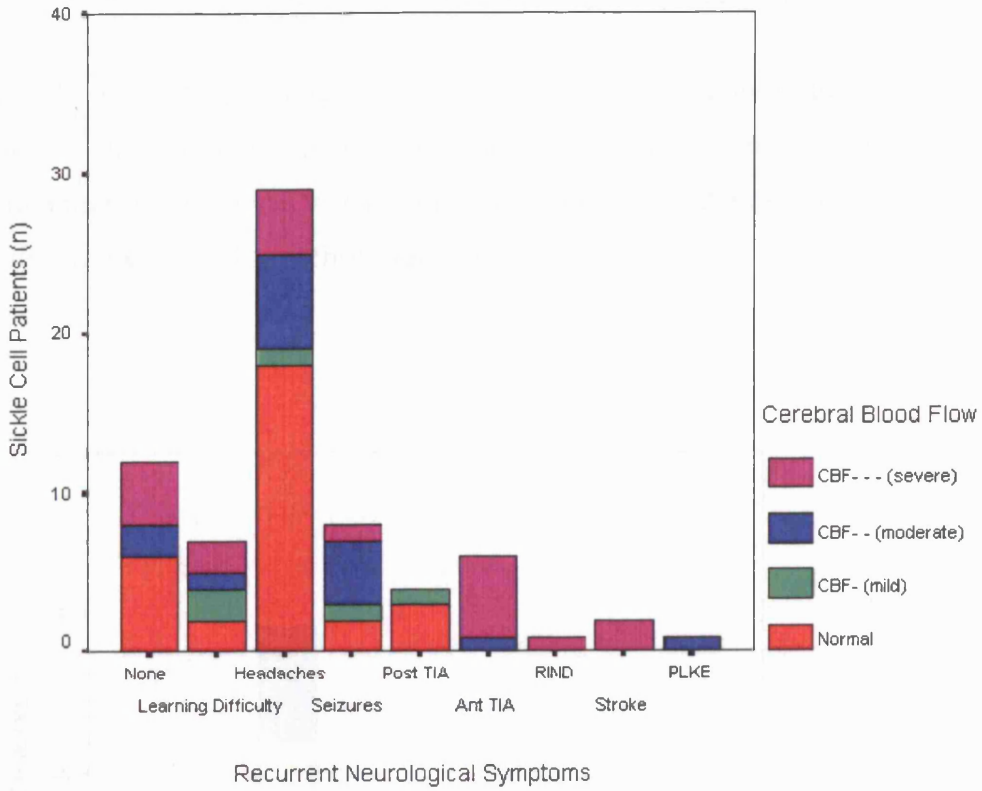


Figure 4.54. Relationship between recurrent neurological symptoms and cerebral blood flow (CBF) maps on perfusion MRI. TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory; RIND= reversible ischaemic neurological deficit.

Recurrent Symptoms (n = Patients)	Normal Perf. MRI (Normal CBF)	Mild Decreased CBF (CBF-)	Moderate Decreased CBF (CBF--)	Severe Decreased CBF (CBF---)	Total
No Symptoms	6		2	4	12
Learning Difficulty	2	2	1	2	7
Headache	18	1	6	4	29
Seizures	2	1	4	1	8
Post TIA	3	1			4
Ant TIA			1	5	6
RIND				1	1
Stroke				2	2
Coma (PLKE)			1		1
Total	31	5	15	19	70

Table 4.31. Relationship between central nervous system events at presentation and cerebral blood flow (CBF) maps on perfusion MRI. TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory; RIND= reversible ischaemic neurological deficit.

4.5.4.2.1. Extent of Perfusion Abnormality and Recurrent Neurological Symptoms

The severity of the recurrent neurological symptoms was not associated with the extent of the perfusion abnormality (p=0.6, Spearman's test). For each recurrent neurological symptom abnormal perfusion was seen in a proportion of patients, either unilaterally or bilaterally (for example, headaches; figure 4.55)

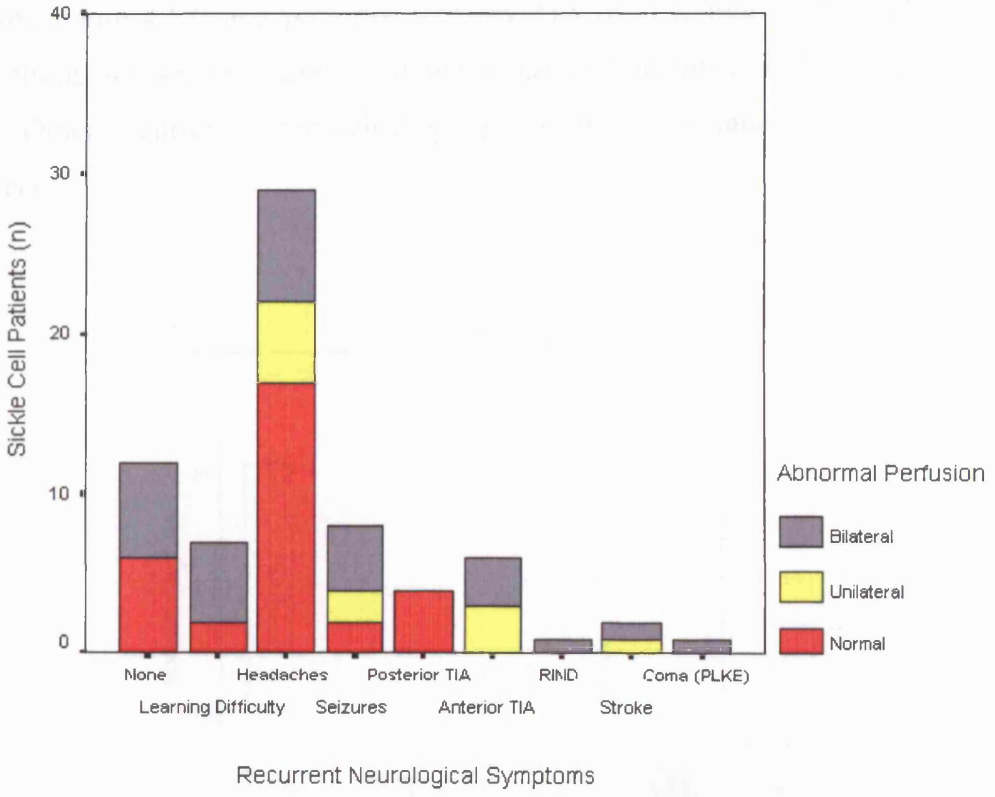


Figure 4.55. Relationship between recurrent neurological symptoms and extension of cerebral perfusion abnormality for each symptom. TIA= transient ischaemic attack, RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy. Unilateral abnormal perfusion= confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI. Bilateral abnormal perfusion= extended to regions of both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s found on MRI.

4.5.4.2.2. Perfusion Abnormality and Recurrent Neurological Symptom

The univariate analysis for the relationship between each recurrent neurological symptom and normal/abnormal perfusion (decreased CBF) showed significant associations for recurrent anterior territory TIA (p=0.001, Mann-Whitney's test; figure 4.56) and headaches (p=0.01; figure 4.57). They were the recurrent neurological symptoms with the most severely decreased CBF and the least severely decreased CBF respectively in this series. There were trends for association for recurrent stroke (p=0.08; figure 4.58) and posterior territory TIA (p=0.1; figure 4.59). There were no associations for seizures (p=0.7; figure 4.60) and learning difficulty (p=0.7; figure 4.61). Other recurrent neurological symptoms were not analysed because of small numbers.

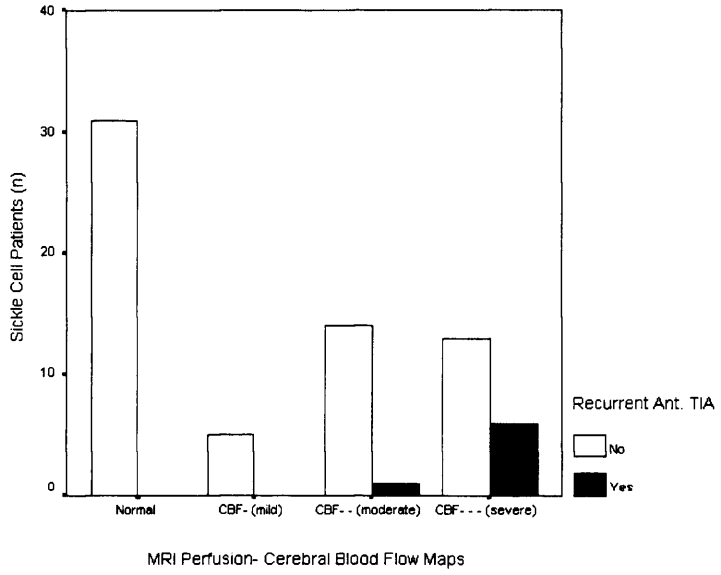


Figure 4.56. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent anterior territory TIA in patients with SCD.

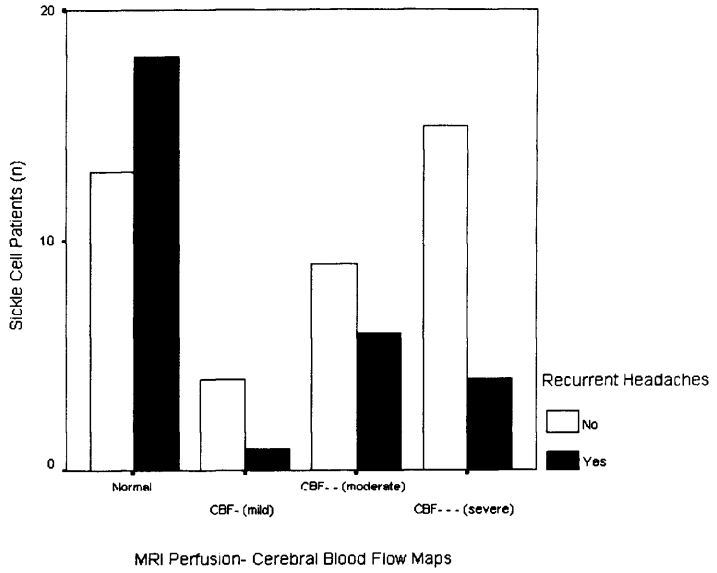


Figure 4.57. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent headaches in patients with SCD.

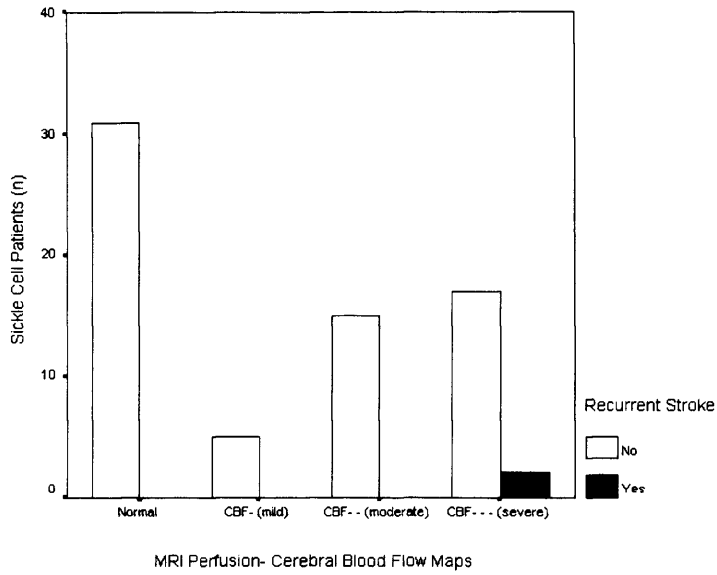


Figure 4.58. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent stroke in patients with SCD.

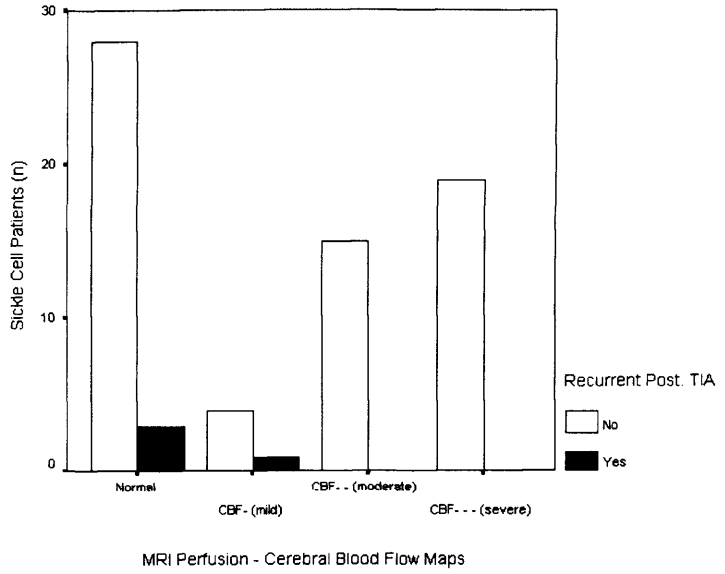


Figure 4.59. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent posterior (post.) territory TIA in patients with SCD.

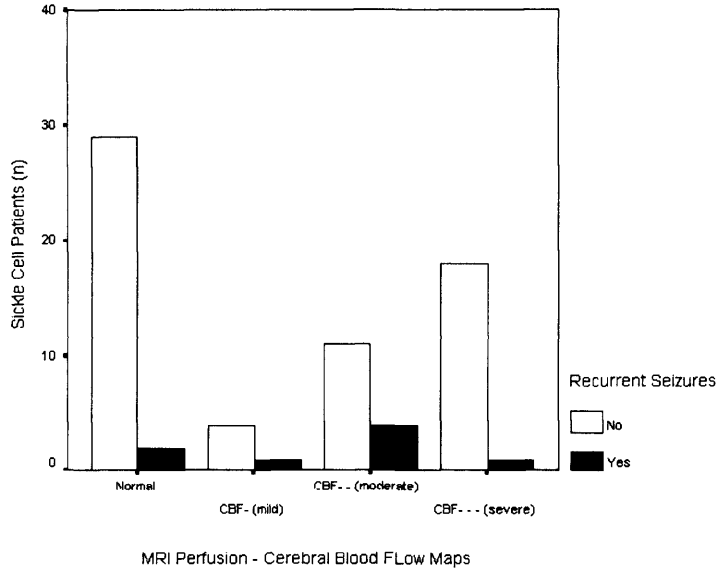


Figure 4.60. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent seizures in patients with SCD.

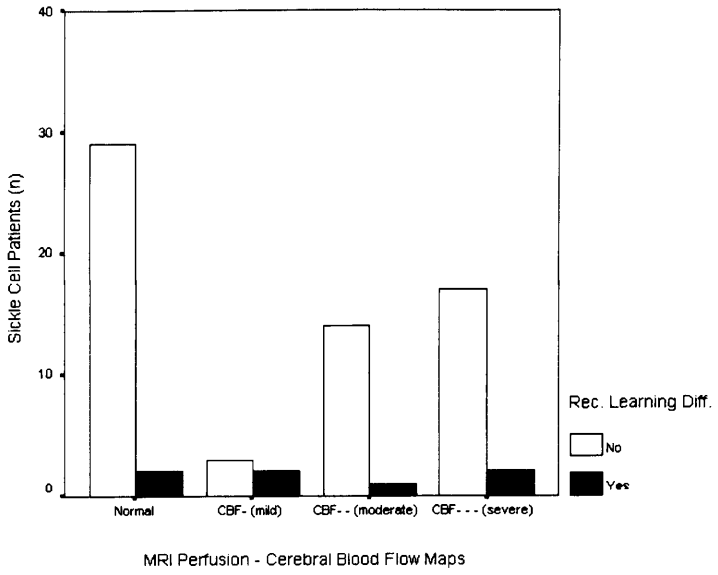


Figure 4.61. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and recurrent (rec.) learning difficulty (diff.) in patients with SCD.

4.5.4.3. Perfusion Abnormality and Parenchymal Imaging (MRI)

4.5.4.3.1. Perfusion Abnormality in Relation with Infarct size and Infarct Number

Worse perfusion abnormality demonstrated by decreased CBF and increased MTT was significantly associated with increased infarct size ($p<0.0001$, CC: 0.6, for CBF and MTT), and increased infarct number ($p<0.0001$, CC: 0.5, Spearman's correlation, for each CBF and MTT) on MRI. Table 4.32 shows the relationship between perfusion MRI abnormality by CBF and infarct size; and table 4.33 shows the association between abnormal perfusion MRI by CBF and infarct number in this series of patients with sickle cell disease.

(n = Patients)	Normal MRI	Infarct < 1cm-small	Infarct 1-5 cm-moderate	Infarct > 5 cm-large	Total
Normal Perf. MRI (Normal CBF)l	27	3	1		31
Mild Decreased CBF (CBF-)	5				5
Moderate Decreased CBF (CBF--)	8	6		1	15
Severe Decreased CBF (CBF---)	3	4	7	5	19
Total	43	13	8	6	70

Table 4.32. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and infarct size.

(n = Patients)	Normal MRA	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	Total
Normal Perf. MRI (Normal CBF)	27				4	31
Mild Decreased CBF (CBF-)	5					5
Moderate Decreased CBF (CBF--)	8	2			5	15
Severe Decreased CBF (CBF---)	3	2	5	1	8	19
Total	43	4	5	1	17	70

Table 4.33. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and infarct number.

4.5.4.3.2. Perfusion Abnormality and Unilateral and Bilateral Cerebral Infarcts

Unilateral infarct was significantly associated with very abnormal perfusion MRI demonstrated by a severe decreased CBF (p=0.005, Mann-Whitney test, figure 4.62) and an increased MTT (p=0.006), whereas bilateral infarcts were significantly associated with severe but also with moderate perfusion abnormality (p=0.001, for decreased CBF [figure 4.63]; and p=0.002 for increased MTT). However, there were unilateral or bilateral infarcts in some patients with normal perfusion MRI (i.e. normal other than in the region of the infarcts themselves).

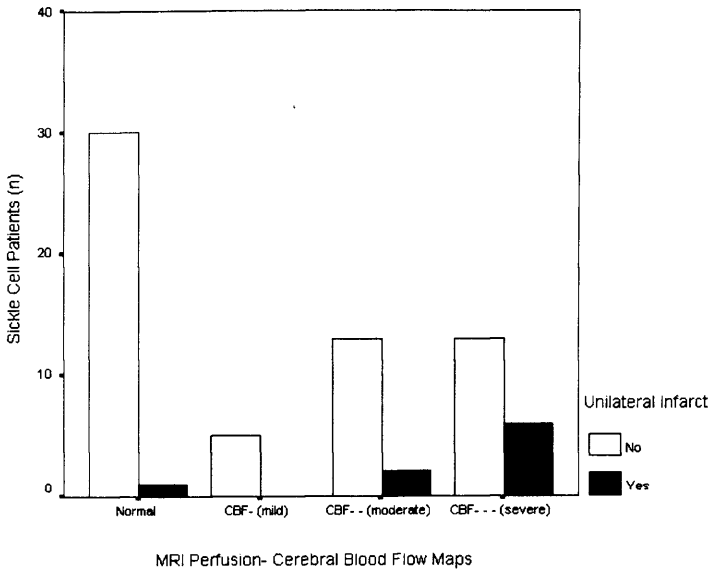


Figure 4.62. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and unilateral infarct.

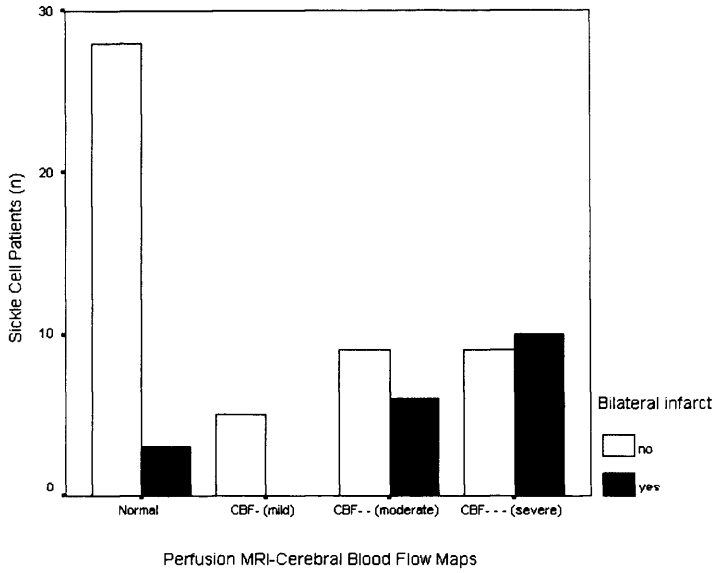


Figure 4.63. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and bilateral infarcts.

4.5.4.3.3. Extent of Perfusion Abnormality in Relation to Infarct Size and Infarct Number

Decreased infarct size and increased infarct number were significantly associated with the extent of perfusion abnormality in both cerebral hemispheres ($p<0.0001$, CC= 0.5 and $p<0.0001$, CC=0.4, Spearman's test, respectively). As discussed in the MRI section, multiple and small/moderate infarcts are more commonly found bilaterally, and were associated with moderate to severe perfusion abnormality as explained above. In addition, unilateral infarcts were also accompanied by a bilateral perfusion abnormality, and there was a large proportion of patients with bilaterally abnormal perfusion and normal MRI.

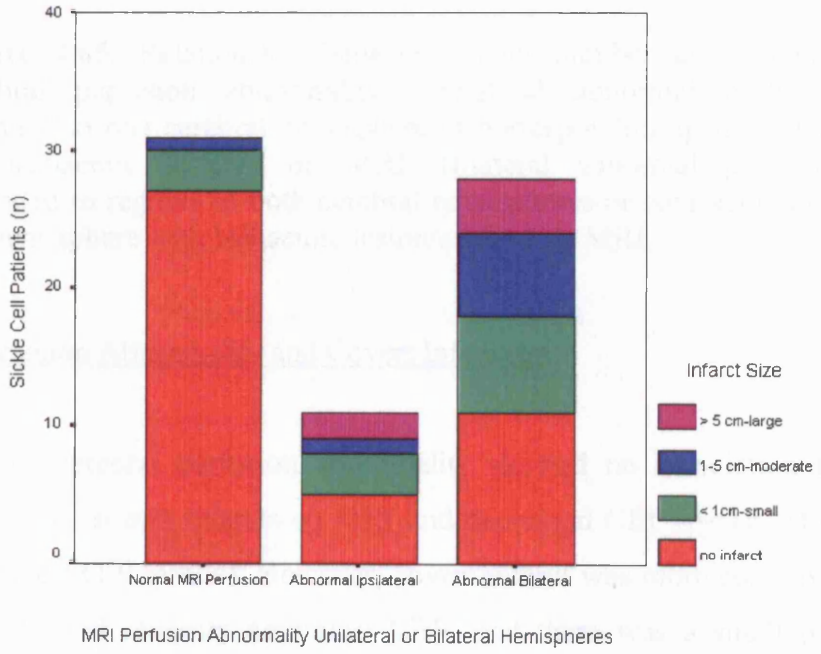


Figure 4.64. Relationship between infarct size and extent of cerebral perfusion abnormality. Unilateral abnormal perfusion= confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI. Bilateral abnormal perfusion= extended to regions of both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s found on MRI.

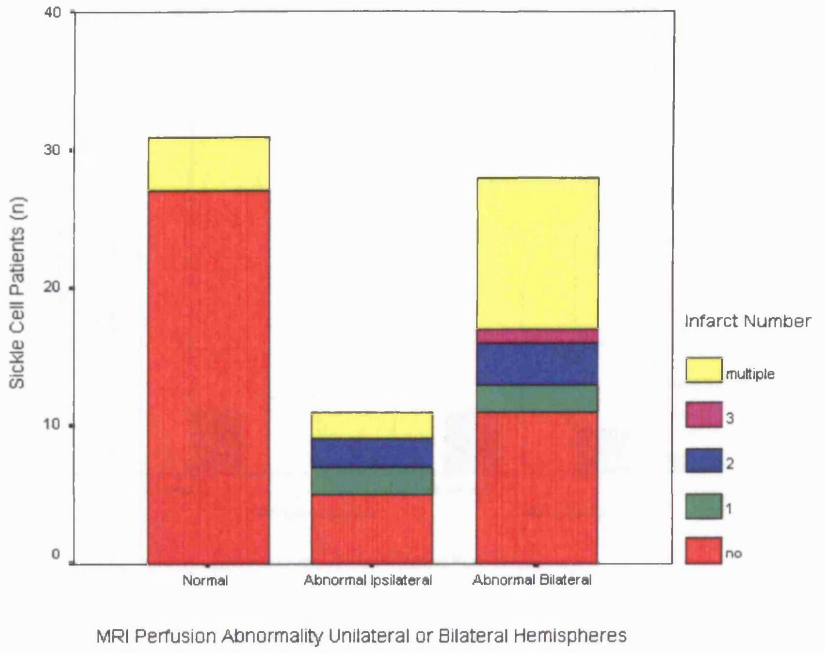


Figure 4.65. Relationship between infarct number and extent of cerebral perfusion abnormality. Unilateral abnormal perfusion= confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI. Bilateral abnormal perfusion= extended to regions of both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s found on MRI.

4.5.4.3.4. Perfusion Abnormality and Covert Infarction

The severity of cerebral perfusion abnormality showed no association between the presence of covert (silent) infarcts on MRI and decreased CBF (p=0.6, Mann-Whitney test) or increased MTT (p=0.4). However, covert infarct was more common in patients with moderately and severely decreased CBF, and there was a small proportion of patients (n=4, [6%]) who had normal perfusion MRI studies and covert infarcts on MRI (Figure 4.66).

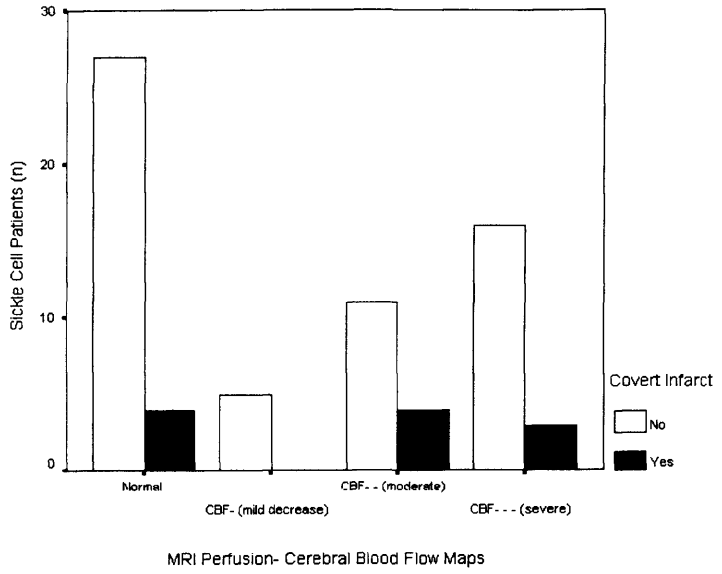


Figure 4.66. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and covert infarcts.

4.5.4.4. Perfusion Abnormality and Magnetic Resonance Angiography

The severity of cerebral perfusion abnormality showed a significant correlation with increased grade of turbulence on MRA ($p<0.0001$, CC= 0.7 for decreased CBF and $p<0.0001$, CC=0.6 for increased MTT, Spearman's test). Figure 4.67 and table 4.34 show this association in this series of sickle cell patients. Furthermore, one third of the patients with normal MRA had abnormal perfusion, characterised mainly by moderately decreased CBF, while a smaller proportion had mildly and severely decreased CBF; there were similar findings in sickle cell patients who had mild turbulence on MRA.

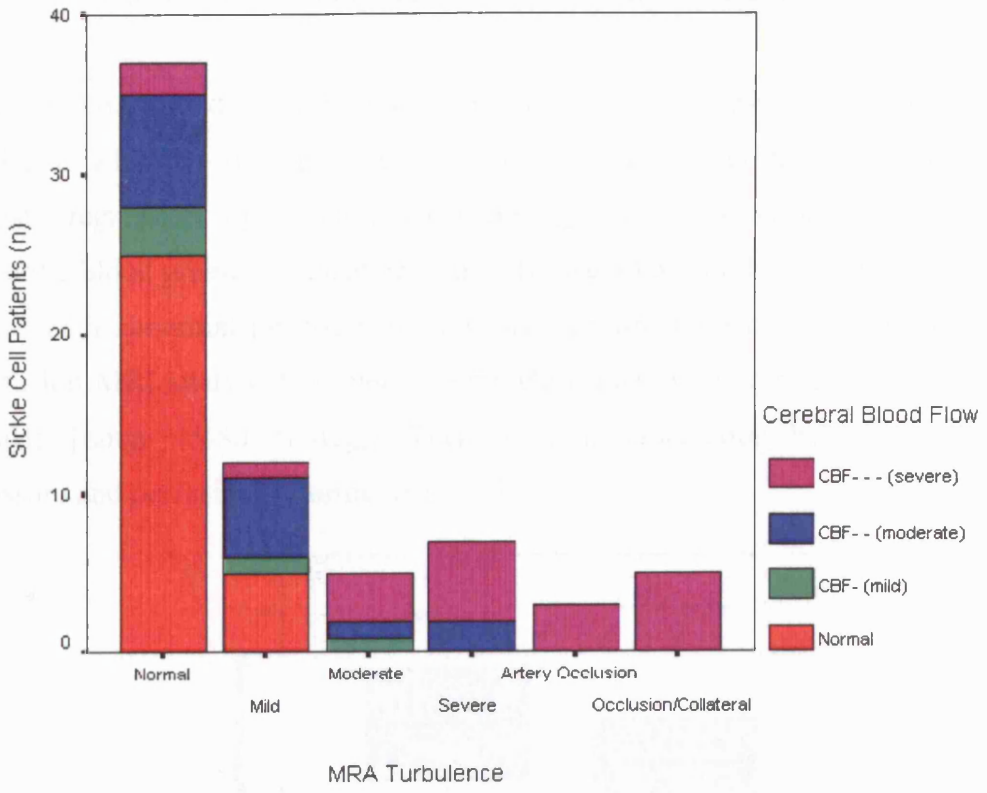


Figure 4.67. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and grades of MRA turbulence.

MRA (n = Patients)	Normal Perf. MRI (Normal CBF)	Mild Decreased CBF (CBF-)	Moderate Decreased CBF (CBF--)	Severe Decreased CBF (CBF---)	Total
Normal MRA	25	3	7	2	37
Mild Turbulence	5	1	5	1	12
Moderate Turbulence		1	1	3	5
Severe Turbulence			2	5	7
Artery Occlusion				3	3
Occlusion + collaterals				5	5
Total	30	5	15	19	69

Table 4.34. Perfusion MRI abnormality (decreased cerebral blood flow –CBF-) and grades of MRA turbulence.

4.5.4.5. Perfusion Abnormality and Blood Pressure Measurements

Mean arterial blood pressure (mean 73.5 mmHg [range 55-95 mmHg]) was lower in patients with abnormal perfusion but this was of borderline significance (p=0.056, logistic regression; figure 4.68), and there was a trend for association for lower mean diastolic blood pressure (mean 55 mmHg [range 30-81 mmHg]; p=0.07, figure 4.69) in those with abnormal perfusion in comparison with those patients who had a normal perfusion MRI study (MAP mean 80 mmHg [range 65-97 mmHg] and DBP mean 62 mmHg [range 45-80 mmHg]). There was no association between systolic blood pressure and perfusion abnormality (p=0.3).

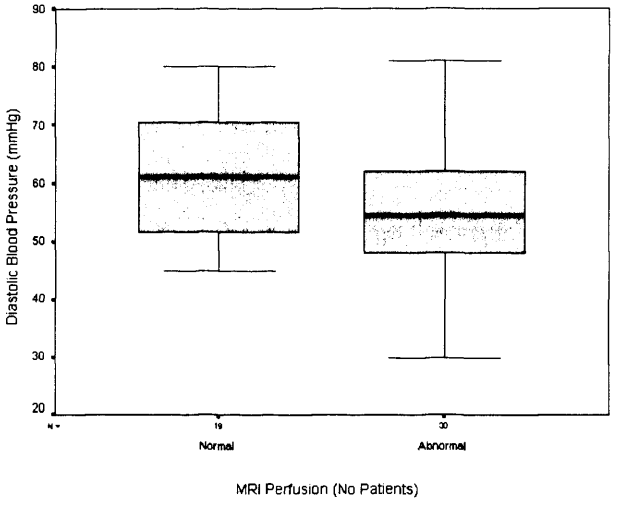


Figure 4.68. Relationship between perfusion MRI abnormality and diastolic arterial blood pressure.

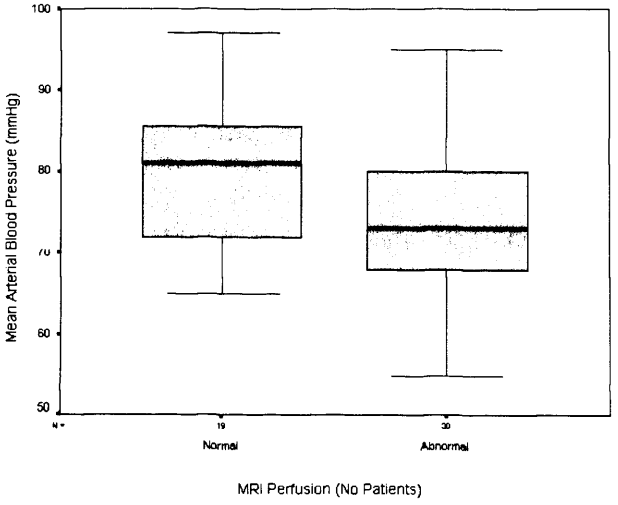


Figure 4.69. Relationship between perfusion MRI abnormality and mean arterial (MAP) blood pressure.

4.5.4.6. Perfusion Abnormality and Oxygen Saturation

There was no association between awake-SpO_2 and the presence or absence of abnormal perfusion (p=0.6, logistic regression).

4.5.4.7. Perfusion MRI Patterns in Relation to CNS Events

Following the data analysis shown in this section, the perfusion MRI pattern for each central nervous system events at presentation in this series of patients with SCD can be summarised as follows (from asymptomatic to most severe neurological symptom, figure 4.50 and table 4.30):

- *No symptoms:* two third of the patients had normal perfusion MRI and one third moderate perfusion abnormality;
- *Learning difficulty:* half of the patients had normal perfusion and half had mild abnormal perfusion;
- *Headaches:* a large proportion of patients had normal perfusion, while smaller proportions had moderate, mild and severe perfusion abnormality;
- *Seizures:* more than half of patients had moderate perfusion abnormality and the rest had normal perfusion;
- *Posterior territory TIA:* more than half of the patients had a normal perfusion, while smaller proportions had moderate, mild and severe perfusion abnormality ;
- *Anterior TIA:* one third of patients had severe perfusion abnormality, while smaller proportions had normal perfusion and moderate perfusion abnormality;
- *Stroke:* mainly severe, while a smaller proportion had moderate perfusion abnormality;
- *Coma:* more than half of the patients had normal study, while smaller proportions had moderate/severe perfusion abnormality.

4.5.3.7. Perfusion MRI Patterns in Relation to Recurrent Neurological Symptoms

The perfusion MRI pattern of this cross-sectional study for each recurrent neurological symptom in this series of patients with SCD can be summarised as follows (from asymptomatic to most severe neurological symptom; figure 4.54 and table 4.31):

- *No symptoms:* half of the patients had normal perfusion MRI, one third severe and a small proportion moderate perfusion abnormality;
- *Learning difficulty* similar proportions of patients had normal perfusion, mild, moderate and severe perfusion abnormality;
- *Headaches:* two third of the patients had normal perfusion, while smaller proportions had moderate, severe and mild perfusion abnormality;
- *Seizures:* more than half of the patients had moderate perfusion abnormality, while smaller proportions had normal perfusion and mild and severe perfusion abnormality;
- *Posterior territory TIA:* mainly normal perfusion, while a smaller proportion had mild perfusion abnormality;
- *Anterior TIA:* mainly severe, while a smaller proportion had moderate perfusion abnormality;
- *Reversible ischaemic neurological deficit (RIND):* severe perfusion abnormality;
- *Stroke:* severe perfusion abnormality;
- *Coma (posterior leukoencephalopathy):* moderate perfusion abnormality.

4.5.5. Transcranial Doppler Ultrasound

4.5.5.1. Transcranial Doppler Ultrasound: Patients and Controls

Sixty-eight patients with sickle cell disease and 37 controls (40 control studies as discussed in the 'Subjects' section of this chapter) underwent non-imaging transcranial Doppler ultrasound. For analysis of the TCD data, the mean maximum velocities of the middle cerebral artery (MCA) and the arterial cerebral artery (ACA) were analysed, comparing controls and patients and by age groups.

Mean maximum MCA (maxMCA) velocity in the patients' group was 94 cm/sec (range 0-230 cm/sec) and in controls was 64 cm/sec (range 30-120 cm/sec). Sickle cell patients had significantly increased mean maxMCA velocity compared to the control group ($p<0.0001$, one-way ANOVA, figure 4.70)

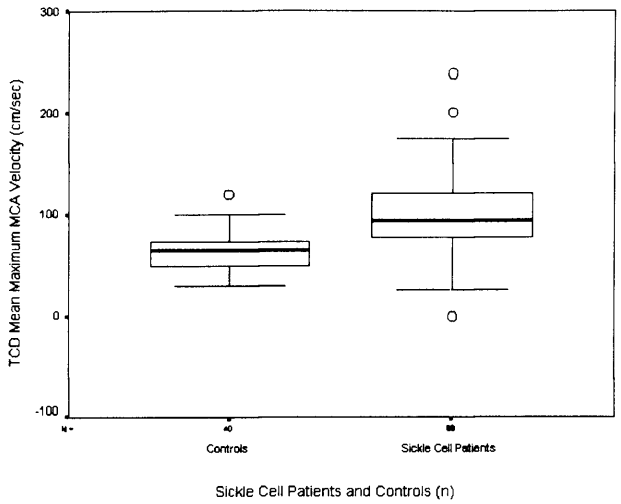


Figure 4.70. Comparison between mean maximum middle cerebral artery (MCA) velocities between controls and patients with SCD.

Mean maximum ACA (maxACA) velocity in the patients' group was 59 cm/sec (range 0-156 cm/sec) and in controls was 69 cm/sec (range 30-120 cm/sec). There was a trend for lower mean maxACA velocities in the sickle cell patients compared with the control group (p=0.1, one-way ANOVA, figure 4.71), however, the highest individual values of maxACA velocities were also found in patients with SCD.

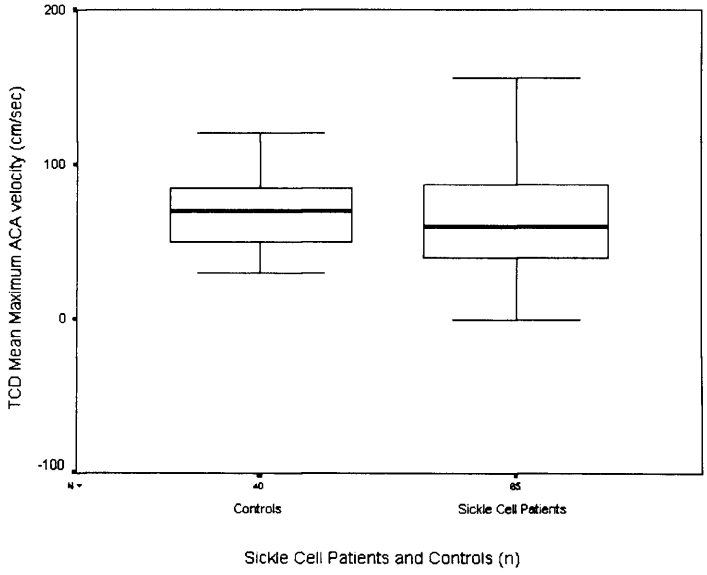


Figure 4.71. Comparison between mean maximum anterior cerebral artery (ACA) velocities between controls and patients with SCD.

The mean maxMCA velocities were significantly different in patients with SCD compared to controls in similar age groups (p=0.003, one-way ANOVA). Post-hoc analysis showed significantly higher TCD velocities in the 6 to 12 years old group compared with the group of 18 to 28 years old (p=0.002; figure 4.72 and table 4.35).

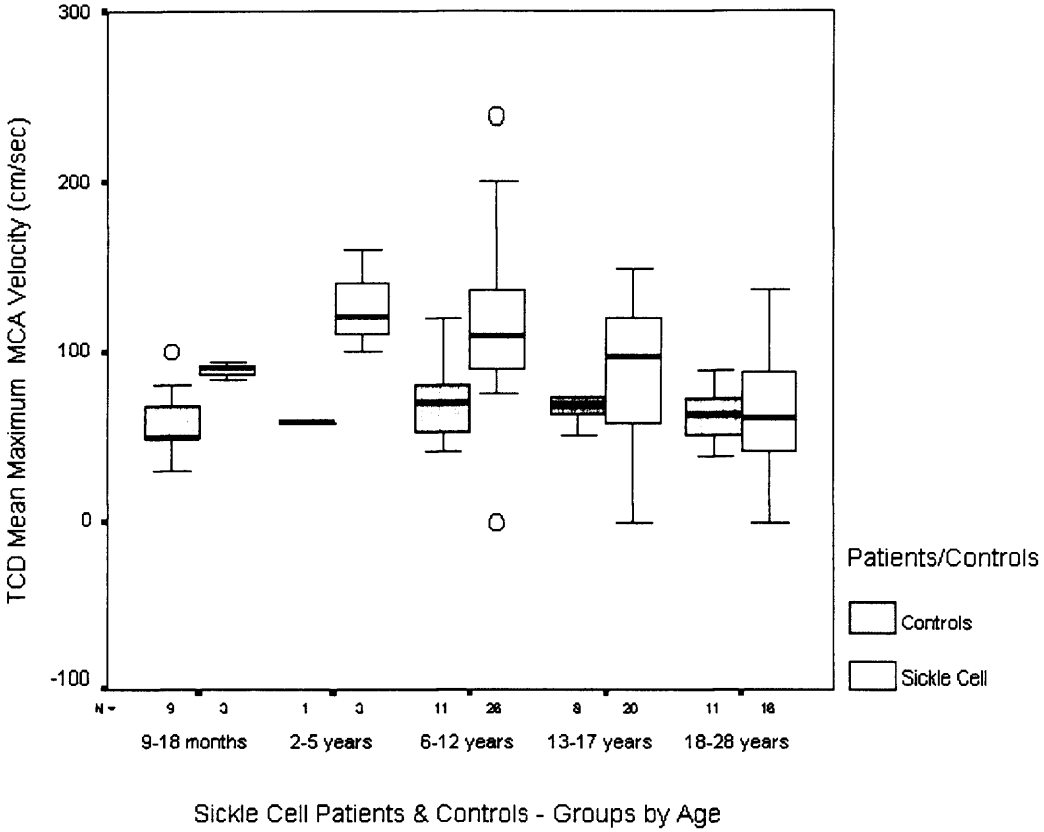


Figure 4.72. Comparison between mean maximum middle cerebral artery (MCA) velocities among age groups between controls and patients with SCD.

Age group	Control Mean Max. MCA (cm/sec)	Control Range Max. MCA (cm/sec)	SCD Mean Max. MCA (cm/sec)	SCD Range Max. MCA (cm/sec)
9-18 months	58	30-100	89	83-94
2-5 years	58	58	127	100-160
6-12 years	69	42-120	115*	0-239
13-17 years	67	51-74	86	0-149
18-28 years	62	38-89	62	0-136

Table 4.35. Mean maximum middle cerebral artery (MCA) velocities among age groups between controls and patients with SCD.*p=0.002 compared to 18-28 year-old group.

In addition, mean maxACA velocities were significantly higher in patients with SCD and controls in relation to their age groups (p=0.01, one-way ANOVA). Analysing separately the patients and the control subjects, both groups had significantly different mean maxACA velocities between age groups (p=0.01 and p=0.028 respectively, one-way ANOVA). In sickle cell patients, post-hoc analysis showed significantly higher maxACA velocities in the 6 to 12 years old group in relation to the group of 13 and 17 years old (p=0.03) and the group of 18 to 28 years old (p=0.018; figure 4.73 and table 4.36). In controls, post-hoc analysis was not performed because of the small numbers, but as is shown in figure 4.55 and table 4.36, the 6-12 year-old controls had higher mean maxACA velocities than the 13-17 year-old and 18-28 years-old groups.

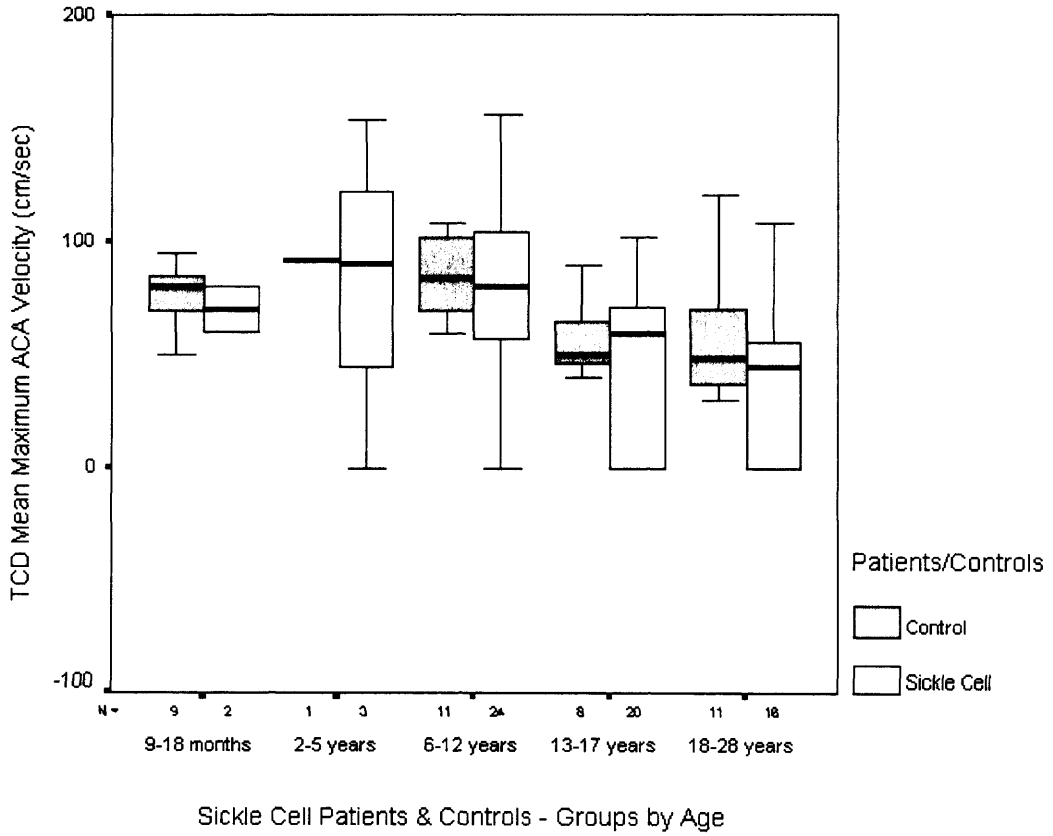


Figure 4.73. Comparison between mean maximum anterior cerebral artery (ACA) velocities among age groups between controls and patients with SCD.

Age group	Control Mean MaxACA (cm/sec)	Control Range MaxACA (cm/sec)	SCD Mean MaxACA (cm/sec)	SCD Range MaxACA (cm/sec)
9-18 months	76	50-95	70	60-80
2-5 years	92	92	81	0-154
6-12 years	84*	59-108	80**	0-156
13-17 years	57	40-90	45	0-102
18-28 years	59	30-120	40	0-108

Table 4.36. Mean maximum anterior cerebral artery (ACA) velocities among age groups between controls and patients with SCD. *p=0.028; **p=0.01 in relation to patients of 13-17 years and 18-28 years of age (one-way ANOVA).

4.5.5.1.1. Comparison between Left and Right Middle Cerebral Arteries and Anterior Cerebral Arteries in Sickle Cell Patients and Controls

There were no significant differences *between sickle cell patients and controls* for the left mean maxMCA (p=0.2, one-way ANOVA) and left and right maxACA velocities (p=0.4 and p=0.2 respectively). There was a trend for higher velocities in the right MCA in the patients compared to the controls (p=0.1, table 4.37).

Sickle cell patients had significantly increased mean maxACA velocities in the left ACA (p=0.001, one way-ANOVA) and right ACA (p=<0.001) compared to the right and left ACAs of the controls.

The comparison between the maximum velocities of right and left MCAs *within patients* showed a trend for higher right MCA velocities (median 89 [0-239] cm/sec) compared with the left (median 87 [0-200] cm/sec; p=0.15, Wilcoxon's test). There were significantly increased left MCA velocities (median 70 [38-89] cm/sec) *within controls,* compared to the right MCA (median 65 [0-87] cm/sec); p=0.02, Wilcoxon's test).

Within patients, there was no difference between the maximum velocities of the left (median 50 [0-154] cm/sec) and right (median 55 [0-156] cm/sec) ACAs (p=0.3, Wilcoxon's test). Similarly *within controls,* there was no difference between the

velocities of the left (median 42 [0-154] cm/sec) and right (median 40 [0-156] cm/sec) ACAs (p=0.2, Wilcoxon's test).

Cerebral Artery	Patients Mean (Median) cm/sec	Patients Range cm/sec	Controls Mean (Median) cm/sec	Controls Range cm/sec
Left MCA	83 (87)	0-200	68 (70)***	38-89
Right MCA	80* (89)**	0-239	60 (63)	0-83
Left ACA	51**** (50)	0-154	42 (42)	0-61
Right ACA	47**** (55)	0-156	33 (40)	0-60

Table 4.37. Comparison between the mean maximum velocities of the left and right middle cerebral artery (MCA) and between the mean maximum velocities of the left and right anterior cerebral artery (ACA) in the sickle cell patients of this series (n=68 for MCA and n=64 for ACA measurements) and controls (n=15 for MCA and n=14 for ACA measurements). *Trend for association compared to *controls* (p=0.1); **p=0.15 compared to left MCA *within patients*; ***p= 0.02 compared to right MCA *within controls;* **** p=0.001 (left ACA) and p<0.0001 (right ACA) compared to *controls.*

4.5.5.2. Transcranial Doppler Ultrasound and Central Nervous System Events at Presentation

Sixty-eight patients underwent transcranial Doppler studies following Adams' methodology and using his criteria as described in chapter 3.

Twenty six (38%) of the 68 sickle cell patients had normal TCD studies, but had presented with the following main CNS events: learning difficulty (n=3), headaches (n=7), seizures (n=5), posterior territory TIA (n=4), anterior territory TIA (n=3), and stroke (n=3). One patient did not have any neurological symptoms.

Of the 42 patients with abnormal TCD, 2 (3%) patients had mean maxMCA velocities more than 200 cm/sec and their CNS events were posterior (n=1) and anterior (n=1) territory TIAs.

Twenty-four patients (35%) patients had decreased mean maxMCA velocities less than 70 cm/sec and ipsilateral MCA lower:higher velocity ratio $\leq$ 0.5 (unilaterally or bilaterally), and their symptoms were headaches in 5, seizures in 1, posterior territory TIA in 7, anterior territory TIA in 3, stroke in 4 and coma in 2. There were no symptoms in 2 patients.

Sixteen patients (24%) had undetectable MCA and they presented with headaches (n=3), seizures (n=1), posterior territory TIA (n=2), anterior territory TIA (n=2), stroke (n=7) and coma (n=1). Figure 4.74 and table 4.38 show the relationship between TCD findings and CNS events at presentation. The severity of the central nervous system events at presentation was significantly associated with TCD category (p=0.004, correlation coefficient [CC]=0.3; Spearman's test).

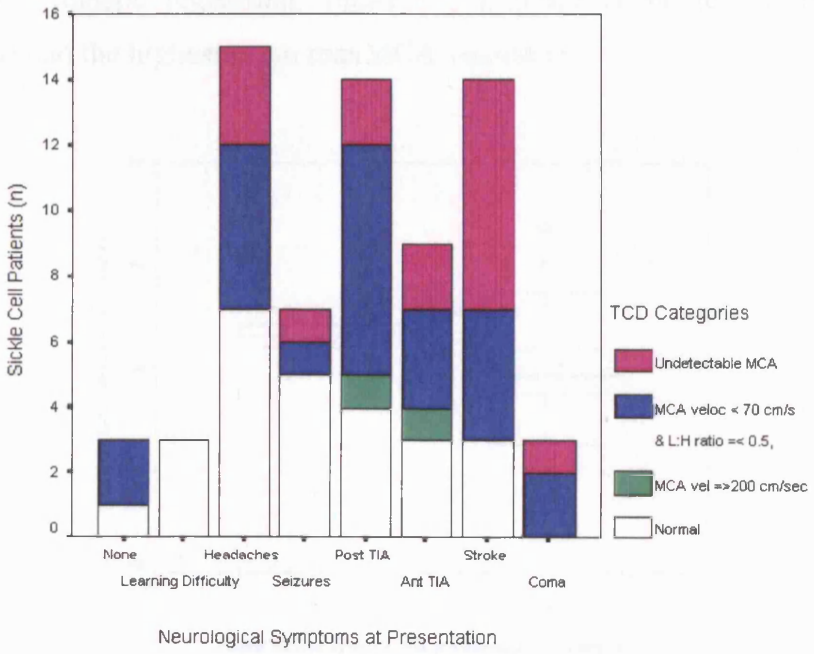


Figure 4.74. Relationship between TCD findings and CNS events at presentation. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity; TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory.

CNS Events (n=patient)	Normal TCD	MCA V =>200 cm/sec	MCA V <70 cm/s & L:H ratio =<0.5	Undetectable MCA	Total
No Symptoms	1		2		3
Learning Difficulty	3				3
Headache	7		5	3	15
Seizures	5		1	1	7
Post TIA	4	1	7	2	14
Ant TIA	3	1	3	2	9
Stroke	3		4	7	14
Coma			2	1	3
Total	26	2	24	16	68

Table 4.38. Relationship between TCD findings and CNS events at presentation. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity (V); TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory.

For the whole group of sickle cell patients who presented with CNS events, there was a trend for association between symptoms and mean maxMCA velocity, independent of age, with lower mean maxMCA velocities (mean 92 cm/s [range 0-230]) in those with symptoms compared with those without symptoms (mean 135 cm/sec [range 121-143 cm/sec], p=0.1, logistic regression; figure 4.75); however sickle cell patients with symptoms also had the highest mean maxMCA velocities.

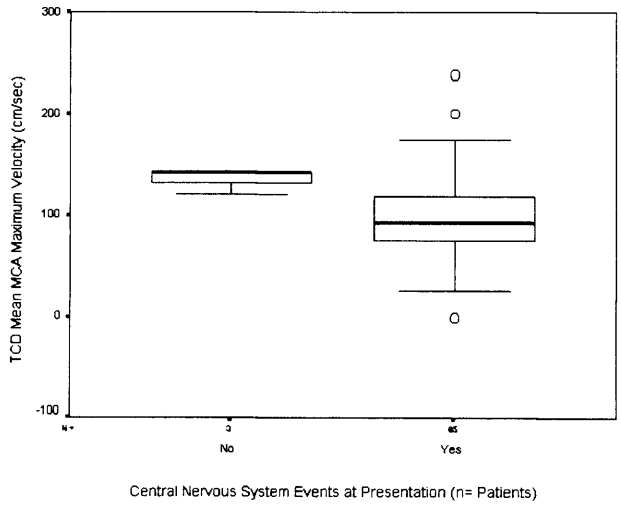


Figure 4.75. Relationship between TCD mean maxMCA velocities and presence or absence of CNS events. MCA=middle cerebral artery

In addition, there was a trend for an association between lower mean maxMCA velocities (mean 87 cm/sec [0-239]) in those sickle cell patients who had stroke, TIA or

seizures compared with those patients who did not have those symptoms (mean 107 cm/sec [0-175 cm/sec], p= 0.1, logistic regression; figure 4.76), although sickle cell patients with more vaculopathy/ischaemia related symptoms also had the highest mean maxMCA velocities.

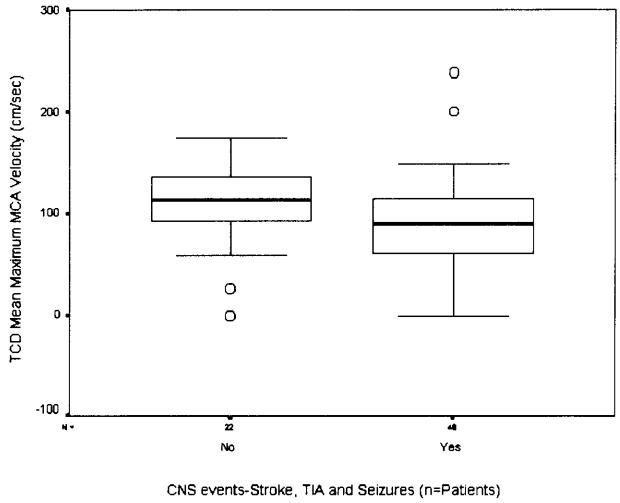


Figure 4.76. Relationship between TCD mean maxMCA velocities and patients who had stroke, TIA and seizures. MCA=middle cerebral artery

For the whole group of sickle cell patients who presented with CNS events, there was a trend for association between mean maxACA velocity and symptoms, independent of age, with lower mean maxACA velocities (mean 58 cm/s [range 0-156]) in those with compared with those without symptoms (mean 109 cm/sec [range 100-117 cm/sec]; p=0.1, logistic regression; figure 4.77), but sickle cell patients with symptoms also had the highest mean maxACA velocities.

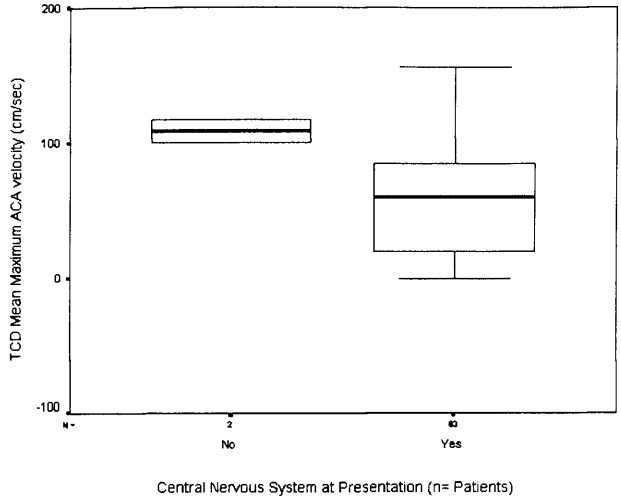


Figure 4.77. Relationship between TCD mean maxACA velocities and presence or absence of CNS events. ACA= anterior cerebral artery.

Furthermore, sickle cell patients who presented with stroke, TIA and seizures had significantly lower mean maxACA velocities (mean 52 cm/s [range 0-156]) than those who did not have those symptoms (mean 74 cm/sec [range 0-154 cm/sec]; p=0.05, logistic regression; figure 4.78).

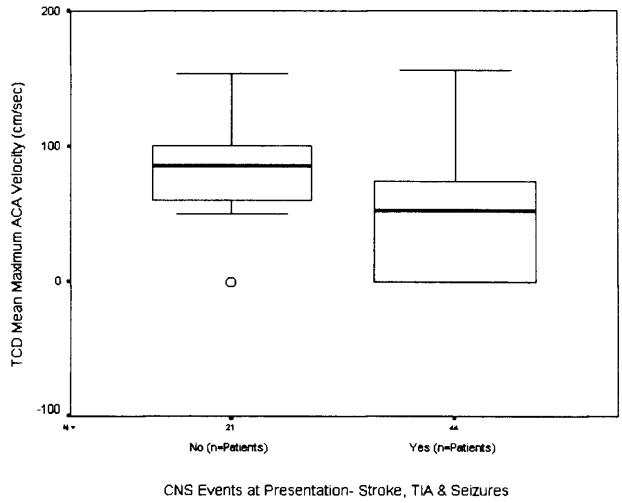


Figure 4.78. Relationship between TCD mean maxACA velocities and patients who had stroke, TIA and seizures. ACA=middle cerebral artery

4.5.5.3. Transcranial Doppler Ultrasound and Recurrent Neurological Symptoms

Twenty six (38%) of the 68 sickle cell patients had normal TCD studies; they presented with the following recurrent neurological symptoms: learning difficulty (n=3), headaches (n=11), and seizures (n=5). Seven patients did not have any neurological symptoms.

Of the 42 patients with abnormal TCD, 2 (3%) patients had mean maxMCA velocities more than 200 cm/sec and their symptoms were recurrent headaches (n=1) and anterior territory TIA (n=1).

Twenty four patients (35%) patients had decreased mean maxMCA velocities less than 70 cm/sec and ipsilateral MCA lower:higher velocity ratio $\leq$ 0.5 (unilaterally or bilaterally), and their symptoms were learning difficulty in 3, recurrent headaches in 11, seizures in 2, posterior territory TIA in 2, anterior territory TIA in 2, and coma with posterior leukoencephalopathy [PLKE] in 1. Three patients were asymptomatic.

Sixteen patients (24%) had undetectable MCA and they had recurrent headaches (n=6), seizures (n=1), posterior territory TIA (n=1), anterior territory TIA (n=3), reversible ischaemic neurological deficit [RIND] (n=1), and stroke (n=2). Two patients remained without symptoms. Figure 4.79 and table 4.39 show the relationship between TCD findings and recurrent neurological symptoms.

The severity of the recurrent neurological symptoms was significantly associated with the TCD category (p=0.007, CC=0.3; Spearman's test).

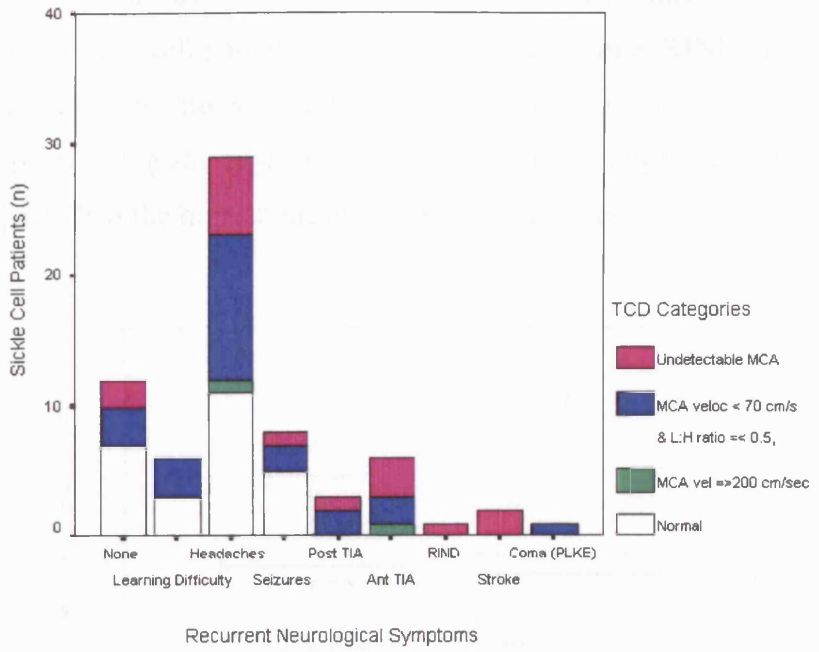


Figure 4.79. Relationship between TCD findings and recurrent neurological symptoms. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity; TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

Recurrent Symptom (n=patient)	Normal TCD	MCA V=>200 cm/sec	MCA V < 70 cm/s & L:H ratio =< 0.5	Undetectable MCA	Total
No Symptoms	7		3	2	12
Learning Difficulty	3		3		6
Headache	11	1	11	6	29
Seizures	5		2	1	8
Post TIA			2	1	3
Ant TIA		1	2	3	6
RIND				1	1
Stroke				2	2
Coma (PLKE)			1		1
Total	26	2	24	16	68

Table 4.39. Relationship between TCD findings and recurrent neurological symptoms. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity; TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy.

There was a trend for lower mean maxMCA velocities (mean 80 cm/sec [0-239 cm/sec]) in those sickle cell patients who had recurrent stroke, RIND, TIA and seizures compared with those patients who did not have those symptoms (mean 99 cm/sec [0-200 cm/sec], p= 0.1, logistic regression; figure 4.80), although one sickle cell patient with anterior TIAs had the highest mean maxMCA velocities .

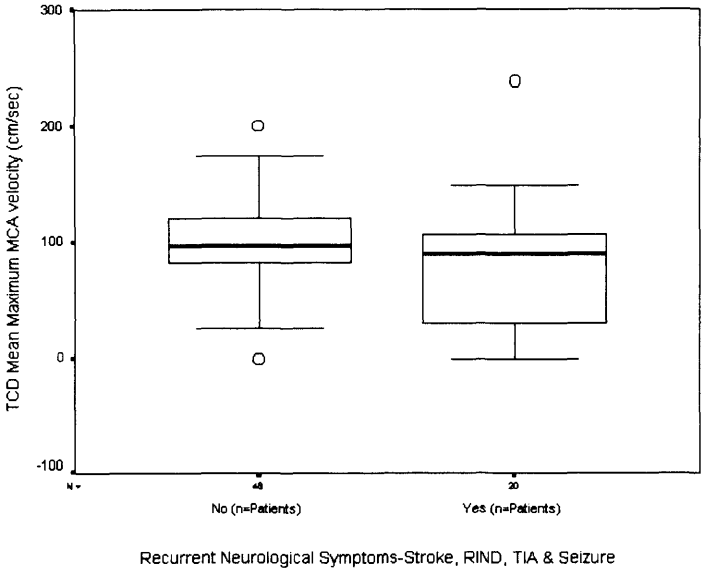


Figure 4.80. Relationship between TCD mean maxMCA velocities and patients who had recurrent stroke, TIA and seizures. MCA=middle cerebral artery

4.5.5.4. Transcranial Doppler Ultrasound and Magnetic Resonance Imaging

The presence or absence of infarction on MRI was significantly associated with increased severity of the TCD category (p=0.001, Mann-Whitney's test). In addition, increased infarct size and infarct number were significantly associated with the grades of severity of the TCD categories (p=0.001 [CC=0.39]; and p<0.0001 [CC=0.42], Spearman's test).

Patients with mean maxMCA velocities less than 70 cm/sec (with lowest: highest ipsilateral MCA velocity ratio $\leq$0.5) or undetectable MCA had larger or more infarcts than the other TCD categories (tables 4.40 and 4.41).

TCD (n=Patients)	Normal MRI	Infarct < 1cm-small	Infarct 1-5 cm-moderate	Infarct > 5 cm-large	Total
Normal	22	2	1	1	26
MCA V =>200 cm/sec	1	1			2
MCA V < 70 cm/s & L:H ratio =< 0.5	12	6	4	2	24
Undetectable MCA	6	4	3	3	16
Total	41	13	8	6	68

Table 4.40. Relationship between TCD findings and infarct size on T2-weighted MRI. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity

TCD (n=Patients)	Normal MRI	1 Infarct	2 Infarcts	3 Infarcts	Multiple	Total
Normal	22	2	1		1	26
MCA V =>200 cm/sec	1		1			2
MCA V < 70 cm/s & L:H ratio =< 0.5	12	1	1		10	24
Undetectable MCA	6	1	2	1	6	16
Total	41	4	5	1	17	68

Table 4.41. Relationship between TCD findings and infarct number on T2-weighted MRI. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity

4.5.5.5. Transcranial Doppler Ultrasound and Magnetic Resonance Angiography

Increased transcranial Doppler ultrasound abnormality was significantly associated with increased turbulence on MRA (p=0.003, CC=0.4, Spearman's test). However, a proportion of sickle cell patients with normal MRA had abnormal TCD studies (undetectable MCA or decreased MCA velocities < 70 cm/sec), perhaps associated with technical difficulties in obtaining an adequate ultrasound signal (thick window) or the age of the patient (whose skull thickness increases over time). On the other hand, a quarter of the patients with vessel turbulence on MRA had normal TCD (figure 4.81 and table 4.42)

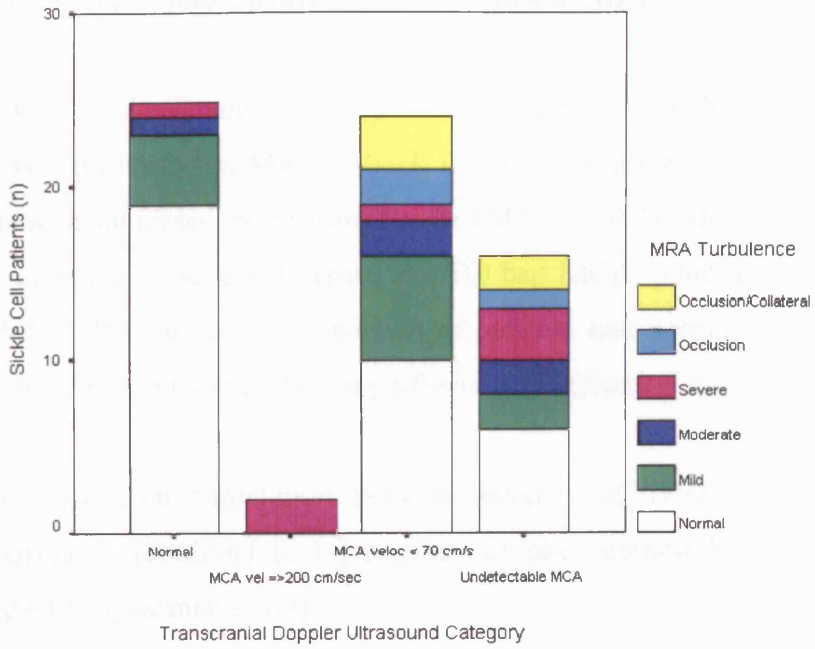


Figure 4.81. Relationship between TCD findings and MRA. MCA=middle cerebral artery; L:H= lowest : highest mean ipsilateral maxMCA velocity.

MRA (n=patient)	Normal TCD	MCA V=>200 cm/sec	MCA V < 70 cm/s & L:H ratio =< 0.5	Undetectable MCA	Total
Normal	19		10	6	35
Mild Turbulence	4		6	2	12
Moderate Turbulence	1		2	2	5
Severe Turbulence	1	2	1	3	7
Artery Occlusion			2	1	3
Occlusion + Collaterals			3	2	5
Total	25	2	24	16	67

Table 4.42. Relationship between TCD findings and MRA. MCA=middle cerebral artery; L:H= lowest: highest mean ipsilateral maxMCA velocity

4.5.5.6. Transcranial Doppler Ultrasound and Perfusion MRI

Transcranial Doppler abnormality was significantly associated with decreased cerebral blood flow (CBF) on perfusion MRI (p=0.03, CC=0.3, Spearman's test) but there was no association with increased mean transit time (MTT, p=0.2). More than half of the patients with moderate or severe decrease in CBF had MCA velocities <70 cm/sec or undetectable MCA. By contrast, a proportion of patients had normal TCD but mildly, moderately or severely decreased CBF on perfusion MRI (figure 4.82 and table 4.43).

There was no significant association between severity of TCD and extent of the perfusion abnormality (confined to [a] region/s of one cerebral hemisphere or both hemispheres; p=0.5, Spearman's test).

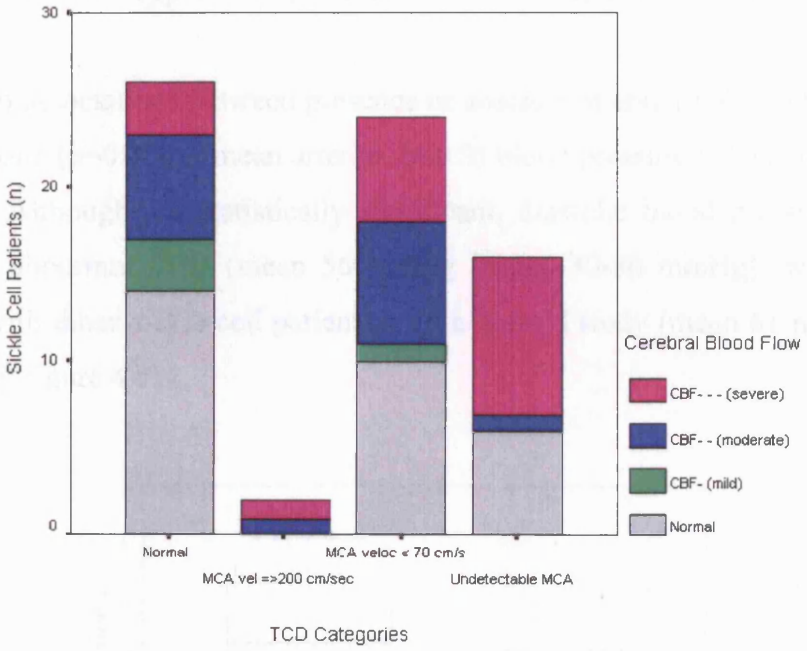


Figure 4.82. Relationship between TCD findings and Perfusion MRI. MCA=middle cerebral artery; L:H= lowest: highest mean ipsilateral maxMCA velocity; CBF: cerebral blood flow.

TCD (n=patients)	Normal Perf. MRI (Normal CBF)	Mild Decreased CBF (CBF-)	Moderate Decreased CBF (CBF--)	Severe Decreased CBF (CBF---)	Total
Normal TCD	14	3	6	3	26
MCA V=>200 cm/sec			1	1	2
MCA V <70 cm/s & L:H ratio =< 0.5	10	1	7	6	24
Undetectable MCA	6		1	9	16
Total	30	4	15	19	68

Table 4.43. Relationship between TCD findings and Perfusion MRI. MCA=middle cerebral artery; L:H= lowest: highest mean ipsilateral maxMCA velocity; CBF: cerebral blood flow.

4.5.5.7. Transcranial Doppler Ultrasound and Blood Pressure Measurements

There were no associations between presence or absence of abnormal TCD and systolic (p=0.7), diastolic (p=0.18) or mean arterial (p=0.3) blood pressure values in these sickle cell patients. Although not statistically significant, diastolic blood pressure values in patients with abnormal TCD (mean 56 mmHg [range 30-80 mmHg]) were lower in comparison with those sickle cell patients with a normal study (mean 61 mmHg [range 33-81 mmHg]; figure 4.83).

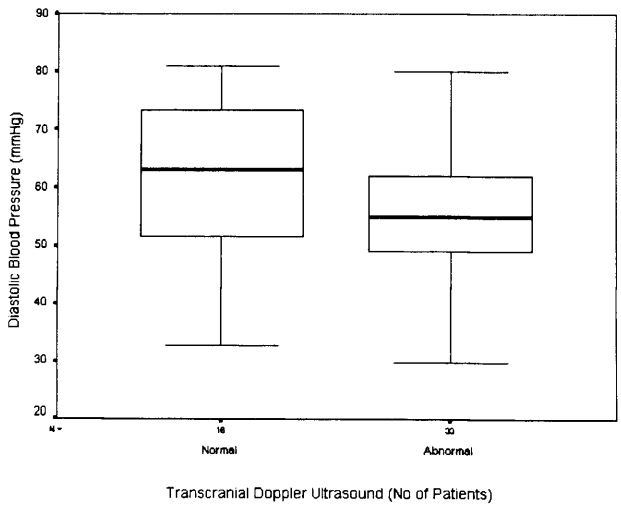


Figure 4.83. Relationship between TCD and diastolic blood pressure measurements.

4.5.5.8. Transcranial Doppler Ultrasound and Oxygen Saturation

There was no association between presence or absence of abnormal TCD and awake-SpO_2 in this series of patients (p=0.6, logistic regression).

4.5.5.9. TCD Patterns in Relation with CNS Events

As the data analysis in this section has shown, the transcranial Doppler ultrasound pattern for each central nervous system event at presentation in this series of patients with SCD can be summarised as follows (from asymptomatic to most severe neurological symptom, figure 4.74 and table 4.38):

- *No symptoms:* two third of the patients had maxMCA velocities <70 cm/sec (unilateral or bilateral) and one third normal TCD;
- *Learning difficulty:* normal TCD;
- *Headaches:* one third of the patients had normal TCD, one third had maxMCA velocity <70 cm/sec, and the remainder had undetectable MCA;
- *Seizures:* two third of the patients had normal TCD, while smaller proportions had maxMCA veloc.< 70 cm/sec and undetectable MCA;
- *Posterior territory TIA:* more than half of the patients had maxMCA velocity < 70 cm/sec, while smaller proportions had undetectable MCA and maxMCA veloc. >200 cm/sec;
- *Anterior TIA:* one third of patients had normal TCD, one third had max MCA velocity < 70 cm/sec, while smaller proportions had undetectable MCA and maxMCA velocity > 200 cm/sec;
- *Stroke:* more than half of the patients had undetectable MCA, followed by maxMCA velocity < 70 cm/sec and normal TCD;
- *Coma:* two third of the patients had maxMCA velocity < 70 cm/sec, while smaller proportions had undetectable MCA.

4.5.5.10. TCD Patterns in Relation with Recurrent Neurological Symptoms

The TCD pattern of this cross-sectional study for each recurrent neurological symptom in this series of patients with SCD can be summarised as following (from asymptomatic to most severe neurological symptom, figure 4.79 and table 4.39):

- *No symptoms:* more than half of the patients had normal TCD, while smaller proportions had maxMCA velocity < 70 cm/sec and undetectable MCA;
- *Learning difficulty:* half of patients had normal TCD, and half had maxMCA velocity < 70 cm/sec;
- *Headaches:* one third of the patients had normal TCD, one third had maxMCA velocity < 70 cm/sec, while smaller proportions had maxMCA velocity > 200 cm/sec;
- *Seizures:* two third of the patients had normal TCD, while smaller proportions had maxMCA velocity < 70 cm/sec and undetectable MCA;
- *Posterior territory TIA:* two third of the patients had maxMCA velocity < 70 cm/sec, while a smaller proportion had undetectable MCA;
- *Anterior TIA:* half of the patients had undetectable MCA, while smaller proportions had maxMCA velocity < 70 cm/sec and maxMCA velocity > 200 cm/sec ;
- *Reversible ischaemic neurological deficit (RIND):* undetectable MCA;
- *Stroke:* undetectable MCA;
- *Coma (posterior leukoencephalopathy):* maxMCA velocity < 70 cm/sec.

4.5.6. Comparison among MRI, MRA, Perfusion MRI and TCD Studies

Comparing the different MR modalities (table 4.44), perfusion MRI was the most sensitive technique to detect cerebral abnormality compared to MRA and MRI (56% vs 46% and 40% respectively) in patients with SCD and neurological complications, with MRA the second most sensitive investigation (figures 4.84 and 4.85).

On the other hand, TCD ultrasound had the highest number of patients overall who had an abnormal study (62%). This high proportion of abnormality surpassed that of

the MR studies; however this may in fact be an artefact of the number of TCDs that were abnormal due to technical difficulties (e.g. thick skull). These technical difficulties, inherent to TCD, might affect in some circumstances the sensitivity and specificity of this investigation in detecting cerebrovascular disease in this population.

Investigation (n=Patients)	**Normal Investigation** n= Patients (%)	**Abnormal Investigation** n= Patients (%)
MRI (n=70)	42 (60%)	28 (40%)
MRA (n=69)	37 (54%)	32 (46%)
Perfusion MRI (n=70)	31 (44%)	39 (56%)
TCD (n=68)	26 (38%)	42 (62%)

Table 4.44. Comparison of normal and abnormal findings in MRI, MRA, perfusion MRI and TCD in sickle cell patients in this cross-sectional study.

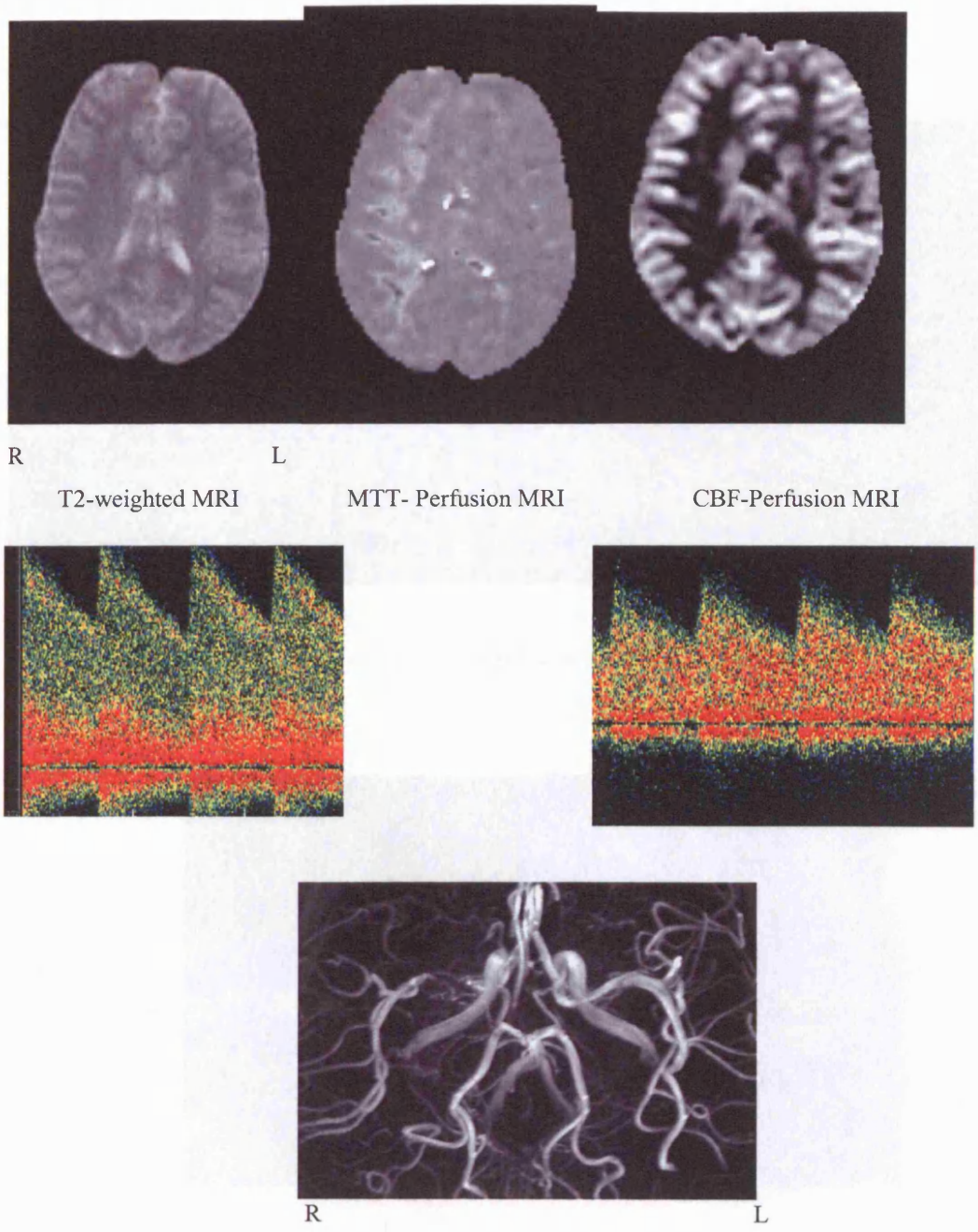
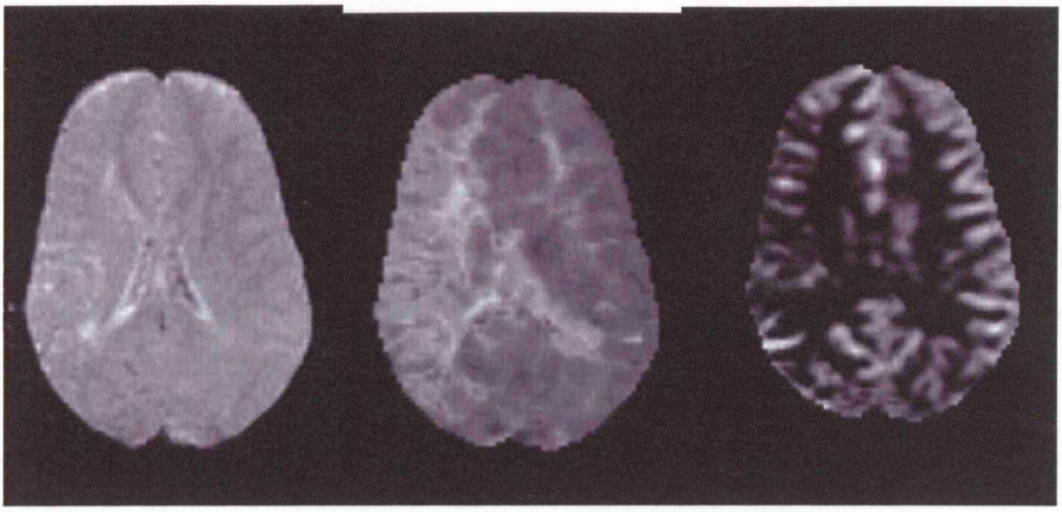
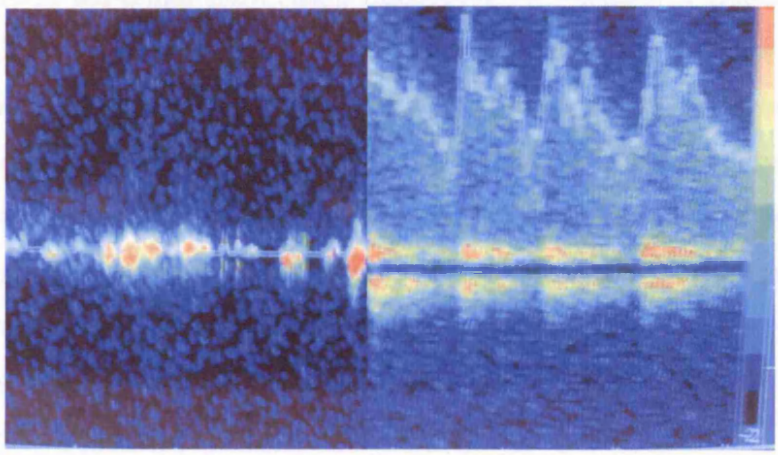


Figure 4.84. Ten-year-old patient with sickle cell anaemia, anterior territory TIA, seizures and headaches, on blood transfusion therapy. ***MRI***: cerebral infarct (subcortical) in the anterior borderzone (MCA/ACA) region on the right. ***Perfusion MRI***: severe increase in the mean transit time (MTT) of the passage of the Gadolinium bolus and decrease in the cerebral blood flow (CBF) in the right deep white matter (MCA/ACA territory). ***MRA***: severe turbulence in the right MCA. ***TCD:*** maximum right MCA velocity: 220 cm/sec, and maximum left MCA velocity: 130 cm/sec.



R L

T2-weighted MRI Perfusion MRI- MTT Perfusion MRI-CBF



TCD: Right MCA TCD: Left MCA

Figure 4.85. Nine year-old patient with sickle cell anaemia who had stroke with left hemiparesis, on blood transfusion. ***MRI***: Ischaemic injury in the right middle cerebral artery (MCA) territory and caudate nucleus. ***Perfusion MRI***: severe increase in the mean transit time (MTT) and decrease in the cerebral blood flow (CBF) in the right MCA territory (cortical/subcortical). ***MRA***: reduced flow in the right terminal carotid artery and right proximal MCA. ***TCD***: severe decreased right MCA velocity (< 70 cm/sec) and normal left MCA velocity (78 cm/sec).

4.6. Results III: Cerebral Perfusion, MRI, MRA, TCD, and CNS Events at Onset and Recurrent Neurological Symptoms by Age Group

There were different patterns of cerebral perfusion, MRI, MRA, TCD, and central nervous system (CNS) events at presentation and recurrent neurological symptoms according to the age groups. Figures 4.86, 4.87, 4.88, 4.89, 4.90 and 4.91 compare these neuroimaging modalities, transcranial Doppler ultrasound and neurological symptoms (at onset and recurrence), which varied among the different ages of the patients of this cross-sectional study. The analysis of the TCD middle cerebral artery velocities by age was described above.

Patterns of extent of the perfusion MRI abnormality for each age group (figure 4.86) were the following: *between 9 to 18 months,* 3 patients had perfusion abnormality in both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s on MRI (bilateral abnormal perfusion); *between 2 to 5 years,* 1 patient had normal perfusion MRI and 3 patients had bilateral abnormal perfusion; *between 6 to 12 years,* 13 patients had normal perfusion, 5 patients had abnormal perfusion confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI (ipsilateral abnormal perfusion) and 9 had bilateral abnormal perfusion; *between 13 and 17 years,* 11 patients had normal perfusion, 3 had ipsilateral abnormal perfusion and 6 had bilateral abnormal perfusion; and *between 18 to 28 years of age*, 6 patients had normal perfusion, 3 abnormal ipsilateral abnormal perfusion and 7 had abnormal bilateral abnormal perfusion.

There were no significant differences in the extent of the perfusion abnormality within the age groups of the 6 to 12 year-old, 13 to 15 year-old and 18-28 year-old sickle cell patients ($p=0.5$, $p=0.2$, and $p=0.6$ respectively; Mann-Whitney test). Younger patients were not analysed because of the small numbers.

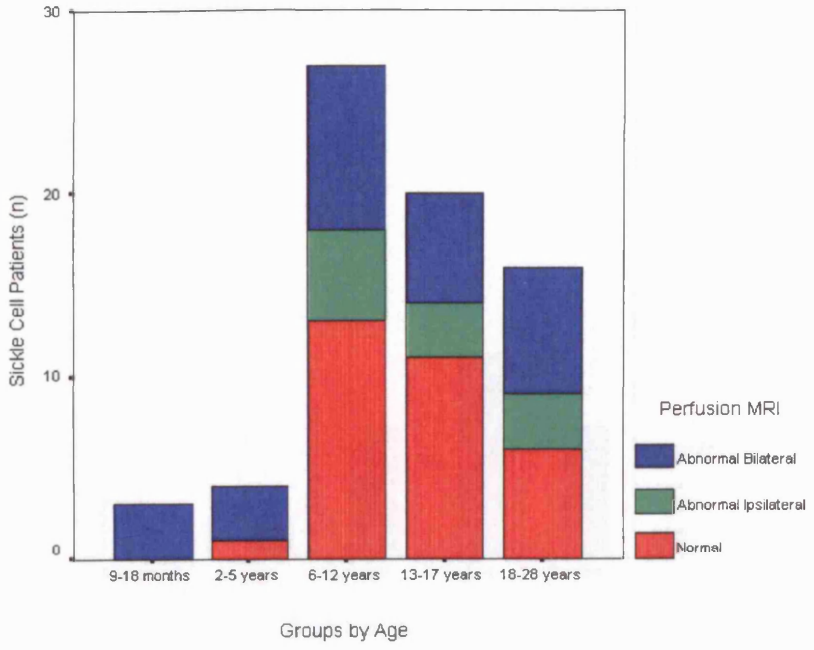


Figure 4.86. Relationship between the ages of patients (by groups) and the extension of cerebral perfusion abnormality. Unilateral abnormal perfusion= confined to one cerebral hemisphere or corresponding ipsilaterally to the ischaemic lesion/s on MRI. Bilateral abnormal perfusion= extended to regions of both cerebral hemispheres or contralaterally to the hemisphere with ischaemic lesion/s found on MRI.

The presence or absence of cerebral infarction on MRI was related to age group as follows (figure 4.87): *9 to 18 months of age,* all the patients (n= 3) had normal MRI; *2 to 5 years*, 2 patients had normal MRI and 2 had infarction; *6 to 12 years*, 20 patients had normal MRI and 7 had infarction; *13 to 17 years*, 9 patients had normal MRI and 11 had infarcts; and *18 to 28 years*, 9 patients had normal MRI and 7 had infarcts.

Within each group, there were trends for a higher proportion of normal MRI studies in the 6 to 12 year-old patients (p=0.1; Fisher's exact test), and for a higher proportion of cerebral infarction on MRI in the 13-17 year-old patients (p=0.1; Fisher's exact test). There were no significant differences between the proportions of either the presence or absence of cerebral infarction on MRI in the 18-28 year-old group (p=0.8). Younger patients were not analysed because of the small numbers.

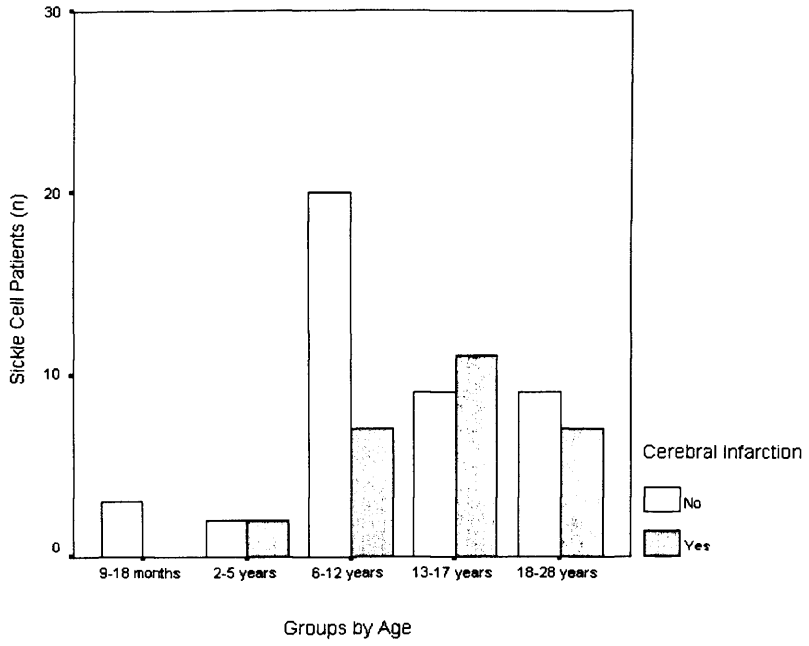


Figure 4.87. Relationship between the ages of patients (by groups) and the presence or absence of cerebral infarction on T2-weighted MRI.

Grades of turbulence on MRA abnormality for each age group (figure 4.88) were the following: *between 9 to 18 months*, 2 patients had normal MRA and 1 had mild turbulence; *between 2 to 5 years*, 2 patients had normal MRA and 2 mild turbulence; *between 6 to 12 years,* 16 patients had normal MRA, 3 mild, 1 moderate and 4 severe turbulence, 1 patient had vessel occlusion and 1 patient had vessel occlusion plus collaterals (moyamoya syndrome); *between 13 and 17 years*, 7 patients had normal MRA, 6 had mild and 3 moderate turbulence, 1 vessel occlusion and 3 vessel occlusion plus collaterals; and *between 18 to 28 years of age*, 10 patients had normal MRA, 1 moderate and 3 severe turbulence, 1 vessel occlusion and 1 vessel occlusion plus collaterals.

There was a trend for a higher number of patients with MRA turbulence in the group of the 13 to 17 year-old (p=0.1; Mann-Whitney test). However, the 6-12 year-old and 18-28 year-old groups did not show significant differences between the number of patients in each age group who had normal studies or turbulence on MRA (p=0.4 and 0.8 respectively; Mann-Whitney test). Statistical analysis was not performed in patients of 5 years of age or less because of the small numbers.

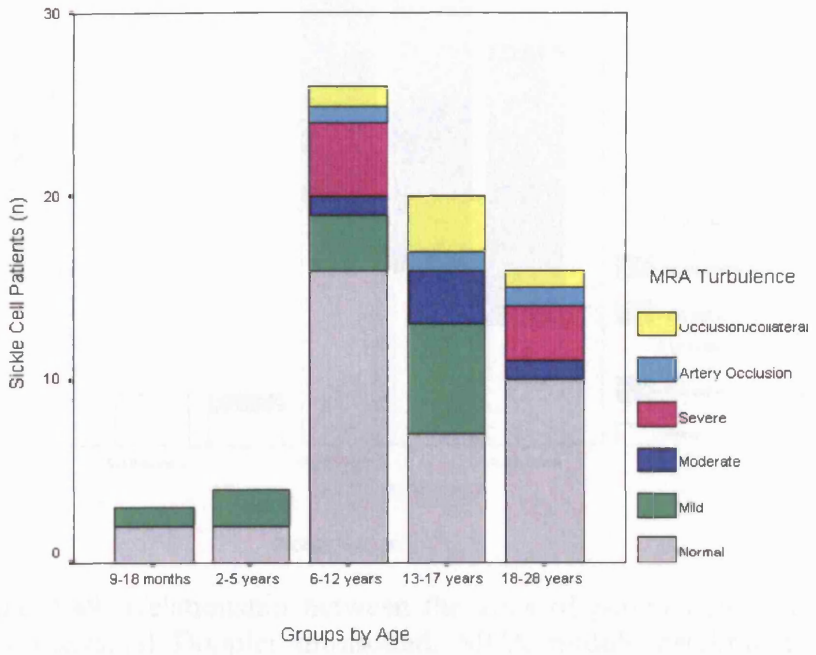


Figure 4.88. Relationship between the ages of patients (by groups) and MRA.

Transcranial Doppler ultrasound finding for each age groups were as follows (figure 4.89): *9 to 18 months of age,* all the patients (n= 3) had normal TCD; *2 to 5 years*, 2 patients had normal TCD and 1 had undetectable MCA; *6 to 12 years*, 14 patients had normal TCD, 2 had maximum MCA (maxMCA) velocities >200 cm/sec, 10 had maxMCA velocities less than 70 cm/sec and lower:higher ipsilateral MCA ratio < 0.5 and 2 had undetectable MCA; *13 to 17 years*, 10 patients had normal TCD, 10 mean maxMCA velocities < 70 cm/sec and 6 had undetectable MCA; and *18 to 28 years*, 7 patients had normal TCD, 8 mean maxMCA velocities < 70 cm/sec and 7 undetectable MCA.

Within each group, patients of 18 to 28 years of age had a significantly increased number of abnormal TCD studies (p=0.046; Mann-Whitney test); and there was a trend for an association for the 6-12 year-old (p=0.085). However, the proportion of normal or abnormal TCD studies was not significantly different for the 13-17 year-old patients (p= 0.5). Younger patients were not analysed because of the small numbers.

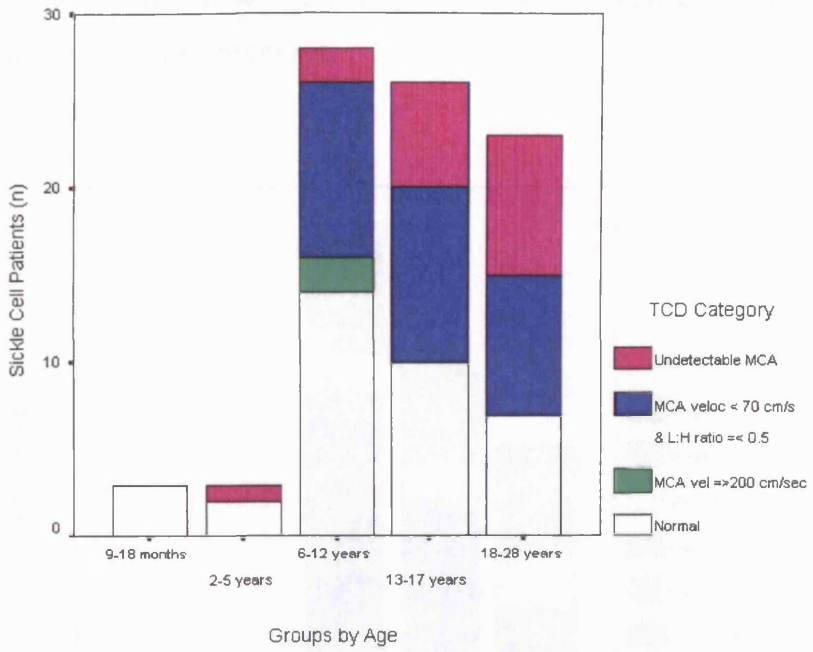


Figure 4.89. Relationship between the ages of patients (by groups) and transcranial Doppler ultrasound. MCA=middle cerebral artery; L:H= lowest: highest mean ipsilateral maxMCA velocity.

The time of the central nervous system events (first event) was not always associated with the time at which the neuroimaging or TCD studies were performed in this cross-sectional series. Some patients had their first CNS events prior to investigation (especially from the group of 18-28 years) whereas other patients had their first neurological symptom after the data had been obtained (age groups of 9-18 months, 2-5 years and 6-12 years). The age groups for CNS events were the following (figure 4.90): *between 9 to 18 months*, 1 patient had generalised tonic-clonic seizures and 2 anterior territory TIAs; *between 2 to 5 years*, 1 patient had learning difficulty, 1 headaches, 1 seizures and 1 stroke; *between 6 to 12 years,* 3 patients had learning difficulties, 6 headaches, 3 seizures, 4 posterior territory TIAs, 2 anterior territory TIAs, 4 stroke, 2 coma and 3 were asymptomatic; *between 13 and 17 years*, 3 had headaches, 1 seizures, 9 posterior territory TIAs, 2 anterior territory TIAs, 4 stroke and 1 coma; and *between 18 to 28 years of age*, 5 patients had headaches, 1 seizures, 1 posterior territory TIAs, 3 anterior territory TIAs, 5 stroke and 1 had coma.

There were no significant differences in the proportion of severe CNS events at onset within each age group of the 6-12, 13-17 and 18-28 year-old patients (p=0.6, p=0.2,

and p=0.3 respectively; Mann-Whitney test). Patients of 5 years of age or less were not analysed because of the small numbers.

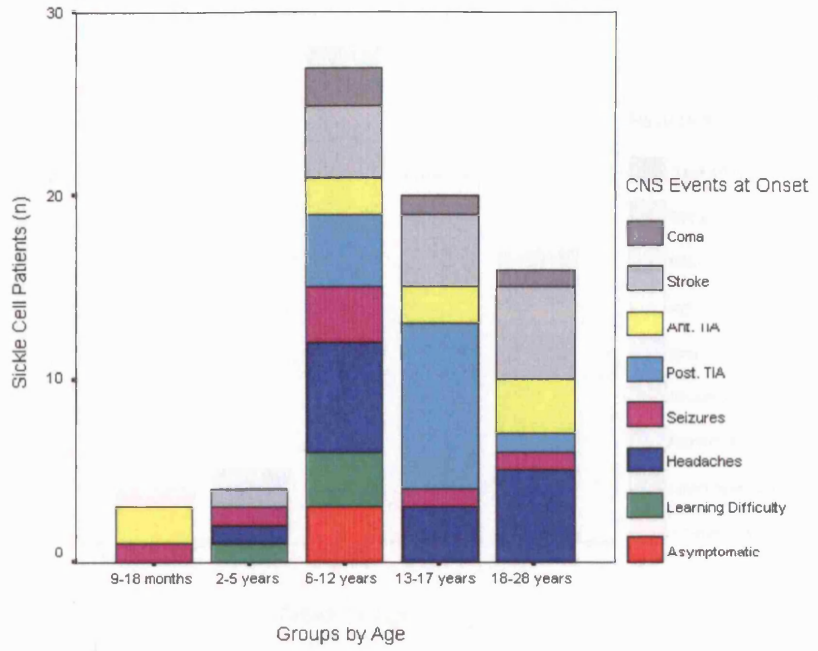


Figure 4.90. Relationship between the ages of patients (by groups) and central nervous system events at presentation. TIA- transient ischaemic attack; ant= anterior territory; post= posterior territory.

The recurrent neurological symptoms observed for each group were the following (figure 4.91): *between 9 to 18 months*, no recurrent symptoms; *between 2 to 5 years*, 1 patient had learning difficulty, 1 headaches, 1 seizures and 1 was asymptomatic; *between 6 to 12 years*, 5 patients had learning difficulties, 8 headaches, 5 seizures, 1 posterior territory TIAs, 3 anterior territory TIAs, 1 coma (posterior leukoencephalopathy [PLKE]) and 4 were asymptomatic; *between 13 and 17 years*, 1 patient had learning difficulty, 12 headaches, 1 seizures, 3 posterior territory TIAs, 2 anterior territory TIAs, and 1 was asymptomatic; and *between 18 to 28 years of age*, 8 patients had headaches, 1 seizures, 1 anterior territory TIAs, 1 reversible ischaemic neurological deficit (RIND), 2 stroke, and 3 were asymptomatic.

The proportion of severe recurrent neurological symptoms within patients of the age groups of the 6-12, 13-17 and 18-28 year-old patients did not reach significance

(p=0.99, p=0.3 and p=0.5; Mann-Whitney test). Younger patients were not analysed because of the small numbers.

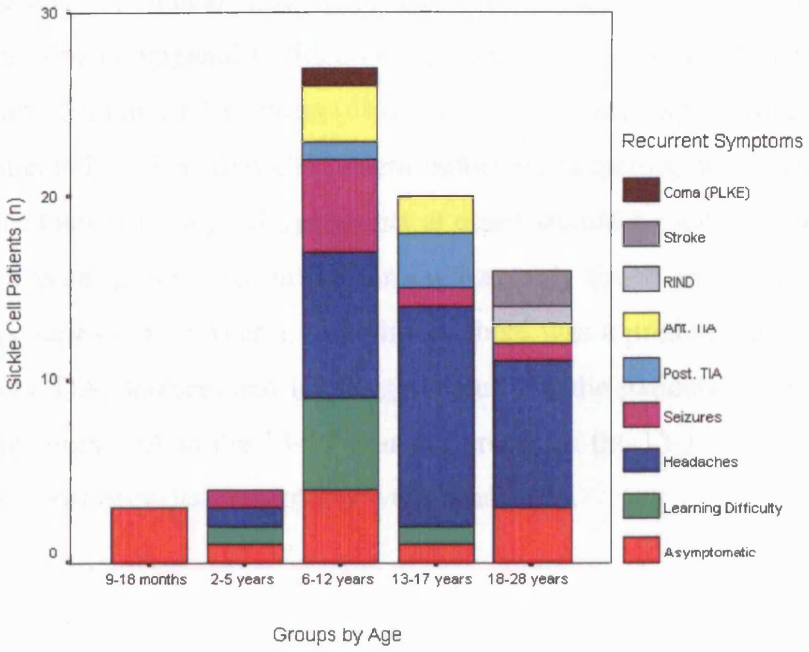


Figure 4.91. Relationship between the ages of patients (by groups) and recurrent neurological symptoms. TIA- transient ischaemic attack; ant= anterior territory; post= posterior territory; PLKE= posterior leukoencephalopathy; RIND= reversible ischaemic neurological deficit.

In this series, as shown in the tables, the prevalence of cerebral infarction increased with age with a peak between 13-18 years, which was related to different grades of MRA turbulence. However, the perfusion abnormalities were more extensive (proportionally) in the younger groups (9-18 months and 2-5 years) in association with a normal or mild turbulence on MRA and with symptoms of seizures and anterior territory TIA. Perfusion abnormality was more localised from 6-12 years of age and onwards; nearly half of those patients had perfusion abnormality either in the same region or in focal regions of one or both cerebral hemispheres.

The age group with greatest prevalence of cerebral infarction on MRI was from 13 to 17 years old (16%), followed by 6-12 years (10%), 18-28 years (10%) and 2-5 years (3%) respectively in this series of patients. However, the TCD data showed that there were patients with critical maxMCA velocities >200 cm/sec only between 6 and 12 years of age, the peak ages for risk of stroke. Another critical period might be very early

in life when some patients presenting with symptoms appear to have extensive perfusion abnormality *without* having severe cerebrovascular disease.

A higher prevalence of clinical stroke at presentation was observed in the 18 to 28 years age group (7%) when compared to the other age groups of 9-18 months (0%), 2-5 years (1%), 6-12 years (6%) and 13-17 years (6%). However, as was explained above, most of these older patients had their first CNS event before participating in this cross-sectional study, therefore their neurological symptoms at onset should be included with the 6-12 years or 13-17 years groups. Recurrent stroke was only found in the oldest group of patients of this series (18-28 years); in addition, there was a predominance of recurrent anterior territory TIA, seizures and learning difficulty in the patients from 6 to 12 years but posterior territory TIA in the 13-17 year-old group. In the 13-17 and 19-28 year-old age groups, the most prevalent symptoms were headaches.

4.7. Results IV: Predictors of Recurrent Neurological Symptoms

Table 4.45 shows predictors of each recurrent neurological symptom in patients with SCD, as found in this cross-sectional study.

The most frequent finding of each neuroimaging modality, transcranial Doppler ultrasound, central nervous system events at presentation, and clinical parameters associated with each recurrent neurological symptom was selected for this table.

The severity of the central nervous system events at presentation was significantly associated with the severity of the recurrent neurological symptoms ($p=0.003$, correlation coefficient [CC]=0.34; Spearman's test).

Recurrent Symptom	CNS Event at Onset	MRI - Infarct Size	MRA Turbul-ence	Perfusion MRI	TCD	Blood Pressure	Awake-SpO_2 %
No Symptoms	Ant. TIA	Normal	Normal	Normal	Normal	NS	NS
Learning Difficulty	Learning Difficulty	Normal	Normal	Normal	Normal	NS	NS
Headache	Headache	Normal	Normal	Normal	Normal	? High SP/ DP	NS
Seizures	Seizures	Normal	Normal	CBF - -	Normal	? High SP/ DP	NS
Post. TIA	Post. TIA	Normal	Normal/ Mild	Normal	MCA <70 cm/sec	? High SP/ DP	NS
Ant. TIA	Ant.TIA/ Stroke	Small	Severe	CBF- - -	Undetec-table MCA	? High SP/ DP	NS
RIND	Stroke	Small	Severe	CBF- - -	Undetec-table MCA	NS	NS
Stroke	Stroke	Large	Occlusion or Occlusion + Collateral	CBF - - -	Undetec-table MCA	NS	? low Awake-SpO_2 %
Coma (PLKE)	Coma	Small	Normal	CBF- -	MCA <70 cm/sec	? High SP	NS

Table 4.45. The most frequent finding of each neuroimaging modality, transcranial Doppler ultrasound, CNS events at presentation and clinical parameters that was associated with each recurrent neurological symptom was selected for this table. TIA= transient ischaemic attack; ant= anterior territory; post=posterior territory; RIND= reversible ischaemic neurological deficit; PLKE= posterior leukoencephalopathy; asymp. = asymptomatic patient; CBF= cerebral blow flow maps; CBF- = mildly decreased; CBF- - = moderately decreased; CBF- - - = severely decreased; MCA= middle cerebral artery; SP= systolic blood pressure; DP= diastolic blood pressure; NS= no significant association.

4.8. Summary of Results

4.8.1. Patients and Neurological Symptoms

- Central nervous system (CNS) events at presentation: 6% coma (n=4 [2/4 patients with stroke and 2/4 with posterior territory TIA]), 20% of sickle cell patients had stroke (n=14 [23% including the 2 patients who had coma and stroke]), 33% TIAs (n=23), 10% seizures (n=7), 21% had headaches (n=15), 6% learning difficulty (n=4) and 4% (n=3) had no neurological symptoms but severe SCD disease.
- Main recurrent neurological symptoms: 3% (n=2) had recurrent stroke, 1% (n=1) had recurrent stroke with coma and posterior leukoencephalopaty, 1% (n=1) reversible ischaemic neurological deficit, 16% TIAs (n=11), 10% seizures (n=7), 44% headaches (n=31), 9% learning difficulty (n=6) and 16% (n=11) had no recurrent symptoms

4.8.2. Clinical Parameters: Blood Pressure Measurements, Haematological Parameters and Oxygen Saturation

- Blood pressure was measured in 49 patients. There were no significant associations between the presence or absence of CNS events or recurrent neurological symptoms and systolic, diastolic and mean arterial blood pressure (BP) values in patients with SCD. However, the patients aged 6 to 12 years had higher blood pressure percentiles.

- Haematological parameters included haemoglobin (Hb), haemoglobin S% (HbS%), white cell count (WCC) and platelets (PTL)
  - *Haemoglobin:* There was a trend for an association between Hb and type of CNS event at presentation (asymptomatic patients had the lowest mean Hb level [7.7 g/d/L], and patients who presented with stroke and coma had the highest mean Hb level [9.7 g/dL and 9.5 g/dL respectively]), whereas there was no significant difference among the mean Hb levels of each recurrent neurological symptoms of the sickle

cell patients. Abnormal TCD was significantly associated with high levels of Hb; and there was a trend for an association between the presence of cerebral infarcts on MRI and high Hb levels. There was no association between Hb level and abnormal MRA. Hb was higher in those with abnormal perfusion but this was not statistically significant.

- *Haemoglobin S%:* Mean HbS% levels of each CNS at onset were significantly different (lower HbS% levels for anterior vascular territory TIA, stroke or coma); but there was no significant difference among the mean HbS% levels of each recurrent neurological symptoms in patients with SCD. Decreased HbS% was significantly associated with abnormal MRA and abnormal perfusion. There was a trend for an association between decreased HbS% and abnormal TCD, but there was no association between HbS% and the presence of infarction on MRI.
- *White Cell Count:* There was no significant association between WCC and the presence of infarction on MRI, abnormal MRA, abnormal TCD and abnormal perfusion MRI. One sickle cell patient who presented with coma and recurrent coma (with posterior leukoencephalopathy) had significantly increased WCC in relation to the WCCs of the patients with other CNS events at onset or recurrent symptoms; however, excluding this patient, there were no significant differences between the mean WCC for the different types of CNS events, and between the mean WCC for each recurrent neurological symptoms.
- *Platelets:* There were no significant differences between the patients' PTL means for each type of CNS event at presentation and recurrent neurological symptoms. In addition, there were no associations between PTL count and abnormal MRI (presence of infarction), abnormal MRA, abnormal TCD and abnormal perfusion MRI.

- Awake- pulse oximetry was measured in 57 patients and was significantly lower in sickle cell patients than in controls (n=14). SpO_2 was not significantly associated with neurological symptoms (at onset and recurrence); however patients with recurrent stroke had lower SpO_2 values in comparison with other recurrent symptoms.

4.8.3. Magnetic Resonance Imaging

- 42/70 patients had normal MRI and 28 patients had an abnormal study (16 overt infarcts, 11 covert infarcts and 1 cerebral atrophy without infarct). 14/28 had cerebral atrophy.
- The severity of the CNS events at presentation was significantly associated with increased infarct size and infarct number, and there was a trend for association between recurrent neurological symptoms and infarct size and number.
- Covert infarction was significantly associated with bilateral location and anterior territory TIA at onset, and there was a trend for association with recurrent anterior territory TIA.
- There was a trend for association between cerebral atrophy and severity of recurrent neurological symptoms, especially with anterior territory TIA, RIND and stroke.
- Lower diastolic blood pressure and MAP were significantly associated with the presence of infarction on MRI. There were no significant associations for systolic BP and awake-SpO_2.

4.8.4. Diffusion Weighted Imaging

- In sickle cell patients with chronic infarction (n=26), DWI was as expected with high diffusion. Only one patient had an abnormal study DWI with restricted diffusion (stroke imaged acutely).

4.8.5. Magnetic Resonance Angiogram

- 37/69 patients had normal MRA and 32 patients had an abnormal study (mild turbulence in 12 patients, moderate turbulence in 5, severe turbulence in 7, vessel occlusion in 3 and occlusion plus collaterals in 5).
- The severity of the CNS events at presentation and the recurrent neurological symptoms were significantly associated with increased turbulence or vessel occlusion on MRA.

- Increased grades of MRA turbulence were significantly associated with the presence or absence of unilateral or bilateral infarcts, covert infarction and infarct size and number.
- Lower diastolic blood pressure and MAP were significantly associated with abnormal MRA. There were no significant associations for systolic BP and awake-SpO_2.

4.8.6. Perfusion MRI

- 31 of the 70 patients had normal and 39 patients had abnormal perfusion MRI (mildly decreased cerebral blood flow [CBF] in 5 patients, moderately decreased CBF in 15, severely decreased CBF in 19).
- The severity of the CNS events at presentation was significantly associated with CBF category and with extent of perfusion abnormality.
- There was a trend for association between recurrent neurological symptoms and the severity of decreased CBF, but not with the extent of perfusion abnormality.
- Worsening of perfusion abnormality was significantly associated with infarct size and infarct number; unilateral infarct on MRI was associated with severely decreased CBF, whereas bilateral infarcts were seen with moderately and severely decreased CBF. Extent of the perfusion abnormality was associated with increased infarct size and number.
- Increased grades of MRA turbulence were significantly associated with the severity of perfusion abnormality.
- A lower MAP was significantly associated with the abnormal perfusion and there was a trend for association with lower diastolic BP. There were no significant associations for systolic BP and awake-SpO_2.

4.8.7. Transcranial Doppler Ultrasound

- Sickle cell patients (n=68) had significantly increased mean maximum MCA (maxMCA) velocities compared to controls (n=40); the group aged 6 to 12 years had significantly higher maxMCA velocities in relation to the older groups.

- There was a trend for an association between lower mean maximum ACA (maxACA) velocities for patients with SCD compared to the control group; however maxACA velocities were higher when compared within groups of patients by age, especially those between 6 to 12 years of age.
- 26/68 patients had normal and 42 patients had abnormal TCD (maxMCA velocities > 200 cm/sec in 2; maxMCA veloc. < 70 cm/sec and lower: higher ratio $\leq$0.5 in 24, and undetectable MCA in 16).
- The severity of the CNS events at presentation and recurrent neurological symptoms were significantly associated with worsening of TCD category following Adams' criteria. Patients (as a whole group) who presented with stroke, TIA and seizures at onset had significantly lower mean maxMCA velocities than those patients who did not have these symptoms, and there was a trend for association between lower maxMCA velocities and patients who had recurrent stroke, RIND, TIA and seizures.
- Worsening of the TCD category was significantly associated with the presence or absence of infarction on MRI and with infarct size and infarct number.
- Increased grades of MRA turbulence were significantly associated with worsening of the TCD category.
- TCD abnormality was significantly associated with worsening of CBF on perfusion MRI but there was no association with extension of the perfusion abnormality.
- Diastolic BP was lower in SCD patients with abnormal TCD but not significantly. There were no significant associations for systolic BP, MAP and awake-SpO_2 and abnormal perfusion.

4.8.8. Cerebral Perfusion Abnormality by Age Group

- The prevalence of cerebral infarction increased with age, predominantly between 13-17 years old.
- Patterns of perfusion abnormality were more extensive in younger symptomatic sickle cell patients in association with a normal or mildly abnormal MRA.

- Perfusion abnormality appeared to be more localised from the 6 to 12 year- old group.
- Critical TCD maxMCA velocities > 200 cm/sec were found only in those in the 6-12 years age group in this series.

4.8.9. Predictors of Recurrent Neurological Symptoms

- Predictors of recurrent neurological symptoms are summarised in table 4.45.

4.9. Discussion

This cross-sectional study showed that sickle cell patients with neurological complications had abnormal cerebral perfusion in more than 50% of the patients. In addition, the cerebral perfusion abnormality had different patterns of severity in relation to the severity of the central nervous system events at presentation of these patients and their recurrent neurological symptoms, and was significantly associated with the severity of the MRI, MRA and TCD abnormalities.

Perfusion MRI (DSC-MRI) appeared to be the most sensitive technique to detect cerebral abnormality with 56% abnormal of the 70 sickle cell patients in this series when compared to the other MR modalities (46% abnormal for MRA and 40% abnormal for MRI).

Decreased mean arterial blood pressure was significantly associated with perfusion abnormality, suggesting that this may be an important factor in lowering cerebral blood flow and contributing to the abnormal perfusion in these patients. As these patients were undergoing chronic blood transfusion, although haematological parameters showed a trend for an association between higher haemoglobin levels and abnormal perfusion, and a significant association between lower Hb S% and abnormal perfusion, no conclusions can be drawn.

Cerebral perfusion has been studied in sickle cell disease over the last two decades in series of small groups of patients in cross sectional studies, using different techniques such as $Xenon^{133}$ (Prohovnik et al 1989), PET (Powars et al 1999), T2*-weighted

(Tzica et al 1993) and perfusion MRI, either dynamic susceptibility contrast MRI -DSC-MRI- (Kirkham et al 2001) using intravenous bolus of Gadolinium, or continuous arterial spin-labelling (ASL) perfusion MRI (Oguz et al 2003).

The series using perfusion MRI had smaller samples of patients with SCD. Oguz et al (2003) only compared ASL perfusion with structural MRI, whereas Kirkham et al, using DSC-MRI, compared different MR modalities (MRI, DWI, MRA) together with transcranial Doppler ultrasound, highlighting the sensitivity of the perfusion MRI over the other investigations (Kirkham et al 2001).

This study focused on identifying patterns of cerebral perfusion abnormality, including its extent and severity, in relation to neurological symptoms, both at initial presentation and at subsequent representation with recurrent symptoms, in patients with SCD. In addition, this study explored the association between different grades of severity in cerebral perfusion abnormality (especially in terms of CBF maps) and the grades of abnormal MRI, MRA and TCD.

This series of patients was necessarily biased in favour of symptomatic patients, in view of the need for intravenous gadolinium. In relation to the central nervous system events at presentation, the prevalence of stroke in this series was higher (23%) compared to a hospital-based cohort which included only patients younger than 19 years (Ohene-Frempong et al 1991) and to population-based studies (Ohene-Frempong et al 1998). However, there were young adults in this study (age range 1 to 28 years) and, interestingly the stroke prevalence was comparable with those series which included adults with SCD reporting a life time risk for stroke between 22 –30% (Ohene-Frempong et al 1991, Platt et al 1994, Styles et al 2000). In addition, more than a quarter of the patients of this study had TIAs (predominantly anterior territory TIA); 10% of the patients had seizures (similar prevalence to that reported by Liu et al 1994), and a very small proportion of the patients were asymptomatic (4%). Therefore, this series of patients was highly symptomatic when compared to other studies (Adams et al 1998 and 2004, Ohene-Frempong et al 1998, Abboud et al 2004), which undoubtedly affects the prevalence of abnormality in the MR and TCD investigations. Although there are currently technical difficulties to be overcome, the use of continuous arterial spin-labelling (ASL) perfusion MRI (Oguz et al 2003) which does not require any

intravenous injection, would allow a population-based study of older children, although it would be difficult to justify sedating asymptomatic younger children for MRI.

Thirty-five of the 70 patients were initially on blood transfusion. However only 20/35 patients remained on chronic transfusion by the time of the investigations; nine patients had stopped blood transfusion therapy and the remainder (n=6) had stopped blood transfusion but continued with alternative treatments (such as Hydroxyurea, bone marrow transplant, or surgical revascularisation procedure plus Hydroxyurea).

The severity of the central nervous system events at presentation was significantly associated with abnormal cerebral perfusion (decreased cerebral blood flow) and the extent of the abnormal perfusion. Stroke was significantly associated with the worst perfusion (severe or moderate decrease in CBF); one third of the patients with anterior territory TIA had also severe perfusion abnormality, whereas headache was the symptom significantly associated with normal perfusion. However, smaller proportions of patients with headaches had mild, moderate and severe decrease in CBF. Learning difficulties and seizures were symptoms with around half of patients with abnormal perfusion (mild and moderate decrease in CBF respectively).

The extent of abnormal perfusion was also related to the severity of the CNS events at onset, so that bilateral (including a region/s of both cerebral hemispheres) perfusion abnormality was found in a higher proportion of patients who had stroke and TIAs at onset; on the other hand, there were also bilateral perfusion abnormalities in around a quarter of patients with seizures, learning difficulties or coma, and in a small proportion of patients without symptoms. Interestingly, 2/4 patients with coma (graded as the most severe symptom), who also had associated symptoms of posterior territory TIA, had normal perfusion MRI studies.

The presence of abnormal perfusion in patients with less severe symptoms such as headaches and seizures (which are not normally considered 'at short-term risk' of developing stroke) showed that, in patients with SCD, some neurological symptomatology (especially severe headaches or seizures) should be considered as 'sentinel' symptoms (Huttenlocher et al 1984, Kirkham et al 2001, Prengler et al 2001 and 2002). Sickle cell patients who have cerebral regions with chronic decreased

perfusion might be at a higher risk of ischaemia (Sorensen and Reimer 2000) if the metabolic rate of the body and regional blood flow demands increase in situations such as infections or sickle cell crisis. Therefore, these patients should be monitored closely in order to prevent severe neurological complications such as stroke.

There was trend for an association between recurrent neurological symptoms and perfusion abnormality. Two patients had recurrent stroke (3%); one patient was on blood transfusion and another had stopped transfusion due to autoantibody formation. Patients with recurrent stroke, recurrent anterior territory TIA (n=6) and reversible ischaemic neurological deficit (n=1) had mainly severe perfusion abnormality (severe decrease in CBF) in their single perfusion MRI study; half of these patients were on blood transfusion, a quarter stopped transfusion (RIND and re-stroked) and the remainder (with recurrent TIA) stopped transfusion but continued with BMT or revascularisation procedure.

On the other hand, a patient who had recurrent coma with reversible posterior leukoencephalopathy in the context of a chest crisis on MRI (he had previously had coma and stroke at onset) and half of the patients who had recurrent seizures had moderate perfusion abnormality (moderate decrease in CBF). Posterior leukoencephalopathy in the context of a chest crisis has been reported in SCD, where an insufficiency of oxygen delivery may explain 'reversible' white matter injury (Henderson et al 2003); in addition leukoencephalopathy in SCD has beeen associated with small blood vessel disease (Steen et al 2003). In addition, recent chest crises have been described as risk factor for stroke (Ohene-Frempomg et al 1998). Seizures in SCD might be a marker for vasculopathy (small and large blood vessel disease) and silent infarction in this population (Adams 1994, Miller et al 1999, Prengler et al 2002).

There was a significant association between recurrent headaches and normal perfusion seen in two third of the sickle cell patients who had this symptom. This might suggest that headache is the least severe symptom in this population, compared to other less severe symptoms such as longstanding learning difficulty or asymptomatic patients who had a variety of grades of perfusion abnormality. However, one third of the patients with headaches had also mainly moderate and severe perfusion abnormality. The presence of abnormal perfusion in patients with these less severe symptoms might be

due to the fact that most of these patients who had learning difficulty, headaches or no symptoms were previously symptomatic patients and were therefore on blood transfusion therapy, which may have prevented the development of more severe recurrent symptoms such as stroke. In addition, headaches have been associated with hyperaemia in SCD (Pavlakis et 1986) and cerebral hyperaemia over time might affect cerebral perfusion (Prohovnik et al 1989), therefore headaches might be associated with impaired cerebral perfusion over time in some patients.

Central nervous system events at presentation were significantly associated with increased infarct size and number and increased turbulence (or increased grade of severity of the cerebrovascular disease) on MR. In turn, increased perfusion abnormality was significantly associated with increased infarct size and number on MRI, and increased turbulence on MRA in patients with SCD.

This study supported previous data associating severity of neurological symptoms (mainly focused on stroke as an end-point) with severity of MRI (Pavlakis et al 1988, Zimmerman et al 1988, Pavlakis et al 1989, Kugler et al 1993, Kirkham et al 2001, Adams 1994, Steen et al 2004) and MRA (Seibert et al 1998, Gilliams et al 1998, Wang et al 1998, Kirkham et al 2002, Abboud et al 2004) abnormality; however, infarct number and infarct size were partially explored in relation to MRA turbulence and perfusion abnormality (Kirkham et al 2001) suggesting an association between the degree of the perfusion abnormality (which extended beyond the area of ischaemic lesions on MRI) and the severity of the cerebrovascular disease in SCD.

The severity of the central nervous system events at presentation was significantly associated with bilateral infarction, increased size of the infarct and increased number of infarcts. Nearly twice as many patients who presented with stroke had bilateral infarction. More than one third of the patients with stroke had severe turbulence, artery occlusion and artery occlusion plus collaterals (moyamoya syndrome), demonstrating the underlying large vessel disease (Pavlakis et al 1989, Stockman et al, Seibert et al 1998). Cerebral perfusion in stroke patients was severely decreased in unilateral infarcts, whereas patients with multiple infarcts had moderately and severely decreased cerebral blood flow. The extent of the perfusion abnormality was associated with the infarct size and number, moreover, the perfusion abnormality extended beyond the

ischaemic lesions seen on MRI in the majority of the sickle cell patients with stroke, demonstrating the presence of tissue at risk and hypoperfused in these patients which could contribute in the genesis of their ongoing recurrent symptoms, a finding which was also reported in the previous study using this technique (Kirkham et al 2001). This study showed that, although most of the stroke patients were on therapy (either blood transfusion or alternative treatments), the perfusion abnormality was still present, perhaps maintaining the 'high' risk of developing a further infarction. This extension of the perfusion abnormality beyond the area of the cerebral infarction, either limited to within a vascular territory (middle cerebral artery [MCA]) or extending to other neighbouring vascular territories (MCA/anterior cerebral artery [ACA]; MCA/posterior cerebral artery [PCA]), showed the role of the progression of the cerebrovascular disease which was not halted or reversed by blood transfusion or other therapies in these patients (Pavlakis et al 1989, Prengler et al 2002, Prengler et al 2003 and 2002).

The recurrent symptoms in the stroke patients were diverse and included recurrent stroke (1/2 had stopped blood transfusion), coma with posterior leukoencephalopathy on MRI and new small infarct on MRI, reversible deficit neurological deficit, anterior territory TIA, headaches and learning difficulty; only three patients remained asymptomatic. Except for two patients who stopped blood transfusion, the others were on transfusion or another type of therapy, demonstrating the extent of the damage beyond the original ischaemic lesion, the progression of the cerebrovascular disease and the extent of the perfusion abnormality, or the way that SCD manifests in each individual ('individual phenotype') may play a role in the outcome ('individual prediction of outcome') of these patients, as Weatherall (1995) suggested.

The prevalence of covert (silent) infarction on MRI in this series of sickle cell patients was 16%, similar to previously reported in children with sickle cell anaemia (17%, in Moser et al 1996) but smaller than other more recent series (older patients), which found a prevalence of silent infarction of around 25% (Miller et al 2001, Saunders et al 2001). In addition silent infarction was significantly associated with TIAs, which was previously reported (Zimmerman et al 1987). Patient with TIAs had small or moderate infarct size.

Patients with anterior and posterior vascular territory TIAs had different patterns on conventional neuroimaging and cerebral perfusion imaging. Anterior territory TIA was characteristic of half of the patients with normal MRI studies and nearly half of the patients with small, multiple covert infarction; MRA was normal in half of the patients, and the remainder had moderate (mainly), mild and severe turbulence on MRA. Perfusion abnormality in two third of the patients was predominantly a severe decrease in CBF, with a smaller proportion of patients with moderate decrease in CBF, with unilateral or bilateral extent of abnormal perfusion in similar proportions, whereas only one third of the patients had normal cerebral perfusion. In the patients with anterior TIAs, mild to moderate turbulence on MRA was related to multiple, small infarcts on MRI, whereas in patients with stroke, the most frequent finding on MRA was severe turbulence or artery occlusion, which was more related to one or multiple moderate-large infarcts; therefore anterior TIA represent symptoms which might precede stroke in relation to the impairment of the perfusion abnormality. The severity of this symptom was reflected in the treatment of this group of patients which was mainly blood transfusion, however one patient had stopped blood transfusion, and another patient stopped blood transfusion but had surgical revascularisation procedure and continued on Hydroxyurea. Nevertheless, patients continued having recurrent TIAs (4/9) or headaches (2/9); only three remained asymptomatic. Therefore, anterior TIA was a severe symptom, which reflected a severe perfusion abnormality and a potential risk factor for further stroke in SCD (Ohene-Frempong et al 1998).

By contrast, patients with posterior territory TIA had milder abnormalities on conventional neuroimaging than those with anterior TIA; two third of the patients had normal MRI, MRA and cerebral perfusion. Only one third of the patients had multiple small infarcts, similar mild turbulence on MRA and moderate decrease in CBF. Patients with posterior TIAs were mainly on blood transfusion therapy, however they continued having recurrent symptoms such as headaches (mainly), recurrent TIAs and seizures, and only one patient remained asymptomatic.

Seizures, together with posterior territory TIA, could be defined as another intermediate neurological symptom in relation to severity and prognosis. Patients with seizures had mainly normal MRI, except for one patient who had cerebral atrophy; in addition MRA was normal in 6 of 7 patients. However, moderate decrease in cerebral blood flow

was found in more than the half of the patients (4/7). Six of the 7 patients who presented with seizures at onset were not on blood transfusion, only two were on anticonvulsants, and the only patient who had received blood transfusion discontinued this therapy. However, 5/7 patients continued having recurrent seizures, a further patient had recurrent headaches and another remained without symptoms, which demonstrated the difficulty in determining the pathophysiology of this symptom (i.e. if the cause is ischaemic, epileptic or both) and in appropriately treating patients with SCD and seizures, as patients on anticonvulsants continued to have recurrent seizures. Four out of the 7 patients had perfusion abnormalities in cerebral regions (Appendix Table 1). It has been suggested that a vasculopathy/ischaemic underlying cause (in addition to the anaemia), could lead to regional cortical perfusion abnormality and seizure genesis (Huttenlocher et al 1984, Adams 1994, Prengler et al 2001 and 2002), and blood transfusion might probably help to relieve this symptom in patients with SCD (see chapter 7).

In this study, symptoms of lesser severity were headaches and learning difficulty. There has been a longstanding controversy over the importance of headaches in SCD because of the uncertainty over whether further investigation and treatment are needed. Headaches have been related to the chronic anaemia in SCD as this symptom improves after transfusion, and patients with SCD and headaches have found to have higher cerebral blood flow than patients without headaches (Pavlakis et al 1986 and 1989). In this study, patients with headaches had mainly normal MRI; only 2 of the 15 patients with headaches had covert (silent) infarction. However, one third of the patients had abnormal MRA with grades of mild, moderate or severe turbulence, and also, abnormal cerebral perfusion was found in a quarter of the patients (4/15) with mild, moderate or severe decrease in CBF. Eleven of 15 patients did not have treatment for SCD, one patient was on Hydroxyurea, and 3 patients were on blood transfusion (one discontinued and another stopped blood transfusion and continued on Hydroxyurea). This group of patients continued with recurrent headaches, only one patient remained asymptomatic. The continuing recurrence of headaches and the abnormal perfusion and CVD, which was seen in a small proportion of these patients, suggest that this symptom might be the first expression of an ongoing process of progression of cerebrovascular disease and cerebral perfusion abnormality, with an accompanying risk of ischaemia.

Learning difficulty was associated with normal MRI and MRA. However, there were perfusion abnormalities, with a mild decrease in CBF, in half of the patients, whereas the other half had normal perfusion. Only one of the 4 patients with learning difficulty was initially on blood transfusion due to a SCD crisis with priapism but he discontinued this treatment (he also had perfusion abnormality on his perfusion MRI). It is of interest that priapism has been associated with cerebrovascular accidents in SCD (Siegel et al 1993) although there are few data on any association with learning difficulty. All the patients in this group had ongoing learning difficulties. However, learning difficulty was the mildest neurological symptom in this series and was associated with the least severe grade of cerebral perfusion abnormality. However, learning difficulty could be a manfestation of underlying chronic ischemia secondary to the anaemia or/and small blood vessel disease as suggested by different studies in sickle cell patients with normal structural MRI and learning difficulties (Steen et al 1998, Steen et al 1999a and 1999b) or with abnormal transcranial Doppler studies (Kral et al 2003).

Finally, those patients who had severe SCD (defined as severe and/or frequent associated symptoms including chest syndrome, priapism or frequent and severe pain crisis) but no neurological symptoms had mainly normal MRI (2/3) and one patient had covert infarction, however two of the patients had abnormal MRA with mild turbulence and one had abnormal cerebral perfusion (moderate decrease in CBF); the remaining patient had normal MRA and perfusion. Only one of 3 patients had treatment with blood transfusion, which was discontinued because she received a bone marrow transplant, and only one patient had ongoing learning difficulty. One patient remained asymptomatic. This small group of patients shows that the brain may be affected in patients without manifesting neurological symptoms.

In relation to MRI and perfusion MRI, the majority of the patients with bilateral cerebral infarction on MRI had bilateral perfusion abnormalities beyond the area of the ischaemic lesion; however, in a small proportion of patients with unilateral infarcts, there were bilateral perfusion abnormalities suggesting that the disease process is affecting regions of both cerebral hemispheres and new areas of the brain are at risk of further ischaemia not seen on structural MRI (Pavlakis et al 1989, Calamante et al 1999 (abstract), Sorensen and Reimer 2000).

MRA and perfusion MRI, although significantly associated, was not comparable in terms of the grading of severity. Perfusion MRI had more severe grades of perfusion abnormalities in relation to the grading of MRA suggesting that the latter imaging technique does not provide exact information on the severity of the cerebrovascular disease and its effect on the cerebral perfusion. Whereas artery occlusion or moyamoya syndrome (artery occlusion plus collaterals) were associated with severe decrease in CBF; mild turbulence on MRA was related to moderate decrease in CBF; and moderate turbulence on MRA to severely decreased CBF in a large proportion of patients. In addition, a quarter of the patients with normal MRA had a moderate decrease in CBF, suggestive of an underlying small blood vessel disease (Pavlakis et al 1988, Pavlakis et al 1989). This finding adds new information about the association of MRA and cerebral perfusion abnormality, whereas previous studies were based on MRA and the presence of cerebral infarction on MRI (Zimmerman et al 1987, Gilliams et al 1998, Seibert et al 1997, Abboud et al 2004).

There were limitations to this study for MRI, MRA and perfusion MRI which are similar because of the visual assessment of these investigations. In the case of MRA, an experienced Neuroradiologist graded the vessel turbulence or occlusion; however there were sometimes small discrepancies in the grading of turbulence intra-observer (when the Neuroradiologist assessed twice the same study). Discrepancies in the MRI assessment were minimal. In the case of perfusion MRI, the author, supervised by a Physicist experienced in this MR technique, assessed the grade of perfusion abnormality using the mean transit time, cerebral blood flow and cerebral blood volume maps of all the patients. However the grading of the CBF maps (decrease in CBF) was associated more significantly with neurological symptoms and the other investigations than the grading of the MTT maps (increase in MTT). This may be because the MTT map appears more highlighted on visual inspection (MTT is white and CBF is dark on the perfusion MRI maps), and for this reason it is more difficult to differentiate grades of severity on the MTT maps than on the CBF maps, which suggests that the CBF map is a very sensitive parameter to detect perfusion abnormality on perfusion MRI in SCD (also discussed in chapter 5).

In relation to transcranial Doppler ultrasound, sickle cell patients had significantly increased mean middle cerebral artery (MCA) and anterior cerebral artery (ACA)

velocities compared to controls, in agreement with previous studies (Brass et al 1988, Prohovnik et al 1989) but there was no significant difference between left or right MCA or ACA within groups. The mean maximum MCA and ACA velocities were significantly increased in children with SCD between 6 and 12 years of age, which is a critical age for stroke in SCD (Ohene-Frempong et al 1991). Increased ACA velocities in TCD have been associated with stroke in children with SCD (Kwiatkwoski et al 2004). In this study, there were trends for association for lower MCA or ACA velocities and patients who had stroke, TIA or seizures, probably because in this series severe cerebrovascular disease (artery occlusion) and associated lower cerebral blow flow were common. On the other hand, there was a wider age range in this study (from 1 year to 28 years) which might affect the normal mean maximum velocities of the MCA and ACA in TCD (higher velocities in children and lower velocities in adults).

The category of the transcranial Doppler ultrasound was significantly associated with infarct size and infarct number, as previously reported (Siegel et al 1995). In addition, the grades of severity on MRA were also significantly associated with the transcranial Doppler ultrasound categories of severity following Adams' criteria (Adams et al 1992, Adams et al 1994). The association between TCD and cerebral angiography (Adams et al 1988) and the association between TCD and MRA have been previously reported (Verlhac et al 1995, Seibert et al 1997), showing that TCD is a useful technique for detecting cerebrovascular disease and the presence of cerebral infarction on MRI. The severity of the TCD category was also significantly associated with the severity of the CNS events at presentation and recurrent neurological symptoms. After the Stroke Prevention Study in Sickle Cell Disease (STOP), the National Institutes of Health recommended prophylactic blood transfusion in those sickle cell patients without stroke who had middle cerebral artery velocities more than 200 cm/sec (Adams et al 1992, Adams et al 1998). Two patients in this series had mean maximum MCA velocities more than 200 cm/sec; one already had had stroke and the other patient presented with frequent headaches, TIAS and seizures, both patients were on blood transfusion therapy and the second patient has not developed stroke during this study, suggesting that this recommendation is probably beneficial (Fullerton et al 2004).

However, the association between TCD and low velocities (less than 70 cm/sec or an ipsilateral mean maximum MCA velocity lowest/highest ratio <0.5) and the risk of

further stroke has been little documented until recently (Minnitti et al 2004). In this study, patients with low velocities in the MCA or undetectable MCA had either normal or different grades of turbulence on MRA (mild, moderate or severe). Patients who had stroke, TIA or seizures (especially stroke and TIA) had lower mean maximum MCA or ACA velocities, probably as an expression of an underlying severe cerebrovascular disease. Moreover, there was a trend for an association between lower MCA and ACA velocities on TCD and recurrent stroke, RIND, TIA or seizures, which showed that lower MCA velocities might also be predictive of recurrent stroke in patients with SCD. However, one third of the patients had low velocities or undetectable MCA on TCD with normal MRA; and also, a quarter of the patients with normal TCD had abnormal MRA (mainly with mild turbulence), which demonstrates that TCD has limitations as a diagnostic tool and could give positive false diagnoses because of the technical difficulties (ultrasound window, i.e. thick skull in SCD secondary to bone marrow changes), the experience of the TCD operator, or the difficulty in reporting an abnormality when the ultrasound signal from the blood vessel is not completely normal, but does not fit the rigorous categories of Adams' (1992) criteria.

Those patients who had abnormal TCD (low velocities or undetectable MCA) and normal MRA (16/35) had recurrent neurological symptoms such as, in those patients with low MCA velocities (8/10): coma with posterior leukoencephalopathy on MRI (n=1), TIA (n=1), headaches (n=5); and, in those with undetectable MCA (5/6): headaches (n=4) and seizures (n=1), which suggests that these patients might have an underlying cerebrovascular disease not detected yet by MRA but identified by TCD (Minniti et al 2004, Steen et al 2004).

Transcranial Doppler was significantly associated with worse perfusion abnormality. More than half of the patients who had low MCA velocities or undetectable MCA had a moderate or severe decrease in CBF on perfusion MRI, demonstrating that an important proportion of patients with low MCA velocities had cerebral regions in the anterior vascular territory at risk of ischaemia. In addition, patients who had MCA mean maximum velocities more than 200 cm/sec had moderate or severe decrease in CBF, confirming Adams' work suggesting that those patients are at risk of stroke (Adams et al 1992, Adams et al 1997). This study has demonstrated that TCD is a good monitor of cerebral perfusion abnormality.

Blood pressure (BP) measurements were not significantly associated with neurological symptoms. Patients of 6 to 12 years had higher blood pressures values that did not reach statistical significance, but this age is at high risk of stroke (Ohene- Frempong et al 1991). Furthermore, 'relative' hypertension in SCD, secondary to factors governing red cell rheology and microvascular tone, has been identified as a risk factor for stroke (Ohene-Frempong et al 1991, Rodgers et al 1993, Pegelow et al 1997). However, lower diastolic blood pressure –DBP- and mean arterial blood pressure -MAP- (analysing the whole group of patients, without taking account of their age) were significantly associated with the presence of cerebral infarction on MRI, abnormal MRA and abnormal perfusion MRI, and there was a trend for an association between lower diastolic blood pressure and abnormal TCD. A study has reported low blood pressure values in patients with SCD (Pegelow et al 1997); however BP varies with age, and in this series, the majority of the patients younger than 17 years had systolic and diastolic blood pressure values between the 50 and 95^{th} percentiles. By contrast, older patients (18-28 years of age) had BP measurements lower than $< 50^{th}$ percentile. Particularly in this age group, lower blood pressure might be a contributing factor to impairment of perfusion abnormality, together with the cerebrovascular disease and this group might contribute to the significant association with the abnormal investigations.

There was an association between high haemoglobin levels and the presence of cerebral infarction on MRI and there was a trend for an association with abnormal perfusion. In addition, low haemoglobin S% level was associated with abnormal MRA and abnormal perfusion MRI. These associations are probably accounted for by those patients who had stroke or TIAs and were on blood transfusion therapy. However, no conclusion can be drawn, as these patients were on chronic blood transfusion to prevent further central nervous system events. In fact, the Cooperative Study of Sickle Cell Disease identified low steady haemoglobin level as a risk factor for ischaemic stroke in SCD (Ohene-Frempong et al 1998), in contrast to the findings in this study; however the data collection of the available haematological data was another limitation of this study, as many of the patients usually had their blood tests taken during post- transfusion or during the sickle cell crisis, and many of the pre-transfusional blood tests were not found during the data collection. However, the effect of increased haemoglobin level

and red cell concentration on the cerebrovascular disease in SCD (especially on small blood vessels) could be explored in future studies.

Although an association between leukocytosis and other blood components involved in inflammation with stroke, covert infarction and cerebrovascular disease in SCD has been reported (Ohene-Frempong et al 1998, Kinney et al 1998, Belcher et al 2000, Prengler et al 2000), there were no associations in this series of patients between white cell count and platelet count with abnormal MRI, MRA and perfusion MRI. However a future study could be done exploring more specifically the association between neutrophils and lymphocytes with the abnormal investigations.

Awake-oxygen saturations were not significantly associated with neurological symptoms, abnormal MRI, MRA, TCD and perfusion MRI; however patients with recurrent stroke had awake SpO_2 less than 92%. Awake- SpO_2 < less than 90% was not associated with stroke risk in adults (Homi et al 1997), however nocturnal hypoxemia secondary to obstructive sleep apnoea has been associated with CNS events in children (Davies et al 1989, Kirkham et al 2001). In fact, mean overnight SpO_2 reflects daytime SpO_2 (Setty et al 2003), although there may additional dips in SpO_2 overnight; the effect of daytime and nocturnal hypoxemia on the risk of recurrent symptoms could be explored in a future study.

Analysing the different neurological symptoms by age groups, in this study it was found the predominant age for cerebral infarction was between 13 to 17 years of age, in contrast to the Cooperative Study of Sickle Cell Disease which identified the highest incidence of stroke in the 2-5 years and 6-9 years of age (Ohene-Frempong et al 1998). However, in this study, cerebral infarction included overt and covert infarction, and covert infarction increases with age and in young adults is around 25% (Miller et al 2001, Saunders et al 2001). However, the age group between 6- 12 years of this series had increased mean MCA velocities (including the critical MCA velocities more than 200 cm/sec), and higher blood pressure percentiles, both predictors of stroke risk. On the other hand, perfusion abnormalities were more extensive (bilateral) in symptomatic sickle cell patients less than 5 years old (the group with highest stroke incidence [Ohene-Frempong et al 1998]), although in this group, one patient had stroke and abnormal investigations, and the remaining patients had a variety of neurological

symptoms such as TIA, seizures, headaches and learning difficulty, predominantly associated with normal MRI and MRA. This could suggest that the perfusion of the developing brain is more sensitive to the abnormal rheology of the sickle cell, especially at the level of the microcirculation and small blood vessels, leading to extensive perfusion abnormalities and more extensive areas at risk of ischaemia, whereas in the 6 to 12 years group and beyond, the perfusion abnormality tends to be more localised. In this study, the highest prevalence of stroke as the CNS event at presentation was in the young adults, however several of these patients had had stroke before these investigation and should be included in the 13-17 year- old group.

Finally, severe perfusion abnormality together with stroke as the CNS event at presentation, severe cerebrovascular disease and undetectable MCA in TCD were predictors of recurrent stroke in this study. Identifying predictors of recurrent neurological symptoms from a single time point investigation in this study showed that the severity of the recurrent neurological symptoms was usually related to the severity of the CNS events at onset, the MR studies and TCD. However, perfusion MRI could detect abnormality in patients with symptoms such as seizure usually associated with normal conventional neuroimaging. Therefore specific CNS events at onset could be delineated, including seizures, posterior TIA, anterior TIA, stroke and coma, which were predominantly symptoms of abnormal cerebral perfusion and predicted risk of further ischaemia and infarction.

4.10. Conclusion

This study showed that sickle cell patients had a high prevalence of cerebral perfusion abnormality. In addition, a distinctive pattern of abnormal cerebral perfusion in association with an abnormal structural MRI and cerebrovascular disease could be recognised, demonstrated by MRA and transcranial Doppler ultrasound, for every neurological symptom in patients with SCD.

The severity of the TCD categories following Adams' criteria was associated with the grades of MRA turbulence or artery occlusion and severity of the perfusion abnormality, which suggests that this non-invasive technique could be a good method of

detecting and monitoring cerebrovascular disease (CVD) and perfusion abnormality in SCD. Moreover, TCD might detect the presence of cerebrovascular disease before MR angiography.

Other clinical parameters, such as low blood pressure, might affect cerebral perfusion in patients with SCD, in conjunction with the underlying CVD.

In this study, perfusion MRI (DSC-MRI) appears to be the most common MRI abnormality in sickle cell patients, in every age group and for each neurological symptom. In addition, perfusion MRI demonstrated that an important proportion of patients with SCD were at high risk of cerebral ischemia and infarction, characteristic of this inherited anaemia and the underlying cerebrovascular disease found in this condition.

Chapter 5: Association of Perfusion Abnormality with Cerebrovascular Disease and Central Nervous System Events in Sickle Cell Disease: A Longitudinal Study

5.1. Introduction and Aims

5.1.1. Introduction

Studies in sickle cell patients have shown that there is an underlying neurological deterioration in a significant proportion of these patients over time. Up to 40% of patients with sickle cell anaemia (SCA) and stroke have recurrent central nervous system (CNS) events despite blood transfusion therapy (Dobson et al 2001). The prevalence of silent infarcts increases with age up around 25% (Miller et al 2001, Saunders et al 2001); and there is a progressive decrease of IQ in children with sickle cell disease as they age, as demonstrated longitudinally in the US Cooperative Study of Sickle Cell Disease (CSSCD) (Wang et al 2001a). The high prevalence of silent infarcts and cognitive deterioration may be markers of progression of cerebrovascular disease over time, which has been well described in earlier pathological studies (Stockman et al 1972) and more recently in longitudinal studies using MRA (Seibert et al 1998). In addition, cognitive deterioration was also associated with chronic anaemia (Steen et al 1999).

Whereas there have been longitudinal studies looking at recurrence of cerebral infarction (overt or covert) or progression of CVD using conventional neuroimaging or transcranial Doppler ultrasound (Adams et al 1992 and 1998), there have been no reports of changes in cerebral perfusion over time, although this might be an earlier and more sensitive marker of cerebral ischaemia in these patients. Most of the published series on cerebral perfusion in SCD have been cross-sectional studies using techniques that involve radioactivity exposure (PET, inhaled Xenon^{133} [Prohnovick et al 1989, Huttenlocher et al 1984, Powars et al 1999]). However, two recent cross-sectional studies using magnetic resonance techniques (dynamic susceptibility contrast MRI [Kirkham et al 2001] and arterial spin labelling [Oguz et al 2003]) have shown

abnormal cerebral perfusion in children with SCD with normal T2- weighted MRI (Oguz et al 2003, Kirkham et al 2001) or beyond the area of cerebral infarction (Kirkham et al 2001). In addition, Kirkham et al (2001) demonstrated cerebral perfusion deficits in association with CNS events and MRA turbulence and abnormal TCD in symptomatic patients with sickle cell disease.

5.1.2. Aim of the Study

The aim of this study was to examine longitudinal MR perfusion imaging findings together with clinical, haematological and conventional MRI and MR Angiogram (MRA) data in patients with sickle cell disease.

The hypothesis to be tested in this study is that in patients with sickle cell disease who have had a central nervous system (CNS) event, there is a progression of cerebral perfusion abnormality associated with progression of cerebrovascular disease (CVD) and with the recurrence of neurological symptoms.

The clinical questions arising from this hypothesis are the following:

1) Is there a worsening of cerebral perfusion over time in patients with sickle cell disease and if so, is it associated with recurrent clinical symptoms?
2) If there is a worsening in cerebral perfusion, is there any correlation with new infarcts or atrophy on MRI?
3) Is any worsening of perfusion abnormality correlated with worsening of cerebrovascular disease (as evidenced by MRA turbulence) over time?
4) Are the changes of patterns of turbulence on MRA (and cerebrovascular disease) correlated with changes in transcranial Doppler ultrasonography over time or recurrent clinical symptoms?
5) Are there haematological factors, including anaemia and leukocytosis, which contribute to the progression of cerebrovascular disease in SCD?
6) Is it possible to identify predictors of recurrent neurological symptoms and future abnormality of perfusion MRI, MRA (progression of cerebrovascular disease) and MRI in sickle cell patients?

2. Subjects

5.2.1. Patients

Data collection for the patients of this study has been partly described in chapter 2 (Appendix Table 1).

Twenty-five patients with sickle cell disease and neurological symptoms were recruited for this longitudinal study. Twenty-two sickle cell patients were recruited from a previous cross-sectional study (Kirkham et al 2001), and they had a second perfusion scan between 2000 and 2003. In addition, three other sickle patients with neurological symptoms were recruited from referrals to joint Haematology/ Neurology clinics attended by the author in six North London hospitals. Therefore, in total, twenty-five sickle cell patients underwent MR resonance and TCD initial and follow-up, and follow-up pulse oximetry (SpO_2) and blood pressure measurements at the Great Ormond Street Hospital for Children NHS Trust (GOSH) and the Institute of Child Health (ICH), UCL. Eight of 22 patients from the previous cross-sectional study were studied in collaboration with Dr Alexandra Hogan, Neuropsychologist, for her PhD project and she supervised their perfusion MRI investigations (between 2000 and 2003). All the patients had sickle cell anaemia (HbSS). Two patients from the earlier cross-sectional study had un-analysable gadolinium perfusion maps at follow-up, and they were excluded from the study. The author supervised the follow-up perfusion MRI studies of 17 out of 23 patients.

Twenty-three patients had a successful MR perfusion study and form the basis of this longitudinal study. All the data analysis was performed by the author. Twelve patients were males and 11 females; the mean age of the patients at the initial scan was 13.4 years (range 6.8 to 22.5 years) and at the final scan the mean age was 15.6 years (range 7.5 to 24.8 years). The patients were followed for a mean of 2.2 years (range 8 months to 3.5 years).

5.3. Methods

5.3.1. Conventional Neuroimaging, Perfusion MRI, TCD, Oxygen Saturation, Blood Pressure Measurements and Haematological Parameters

The patients underwent initial and follow-up MRI, MRA, DWI, and perfusion MRI studies following a published protocol (Kirkham et al 2001), transcranial Doppler ultrasound at onset (n=17) and follow-up (n=25) and follow-up pulse oximetry (SpO_2, 20/25), with a median of 3 years (range 1 to 4 years). Measurement of blood pressure (usually after the perfusion MRI) was done in every patient at follow-up, unless there were technical reasons or the perfusion study was not supervised directly by MP (Dr Hogan's cohort). The data acquisition for the perfusion investigations is described in chapter 3. In addition, the available haematological data were collected from the clinical records of the patients.

5.3.2. Analysis of the Data

For the analysis of the data of the longitudinal study, the main outcome measures were changes shown by neuroimaging (MRI and MRA) and studies of cerebral blood flow (perfusion MRI and TCD) over time. These changes were defined as improvement, no change or worsening of the relevant parameters (see below for how these were categorised). The recurrence of neurological symptoms was also documented. Therefore, the primary data analysis focussed on changes in the patients from either normal or abnormal baseline studies in relation to their symptoms and cerebral perfusion. It was assumed that it was impossible to demonstrate improvement from either a normal or from an abnormal structural MRI (i.e. that evidence of infarction would not disappear), or from normal MRA, TCD and perfusion MRI.

Recurrent neurological symptoms were analysed in two ways, firstly as ordinal data in increasing order of symptom severity (0= asymptomatic; 1= cognitive problems [learning/behaviour]; 2= headaches; 3= seizures; 4= posterior territory TIA; 5= anterior territory TIA; 6= reversible ischaemic neurological deficit [RIND]; 7= stroke; and 8=

coma (posterior leukoencephalopathy [PLKE]), and secondly as binary data in relation to an outcome (recurrence or not of neurological symptoms).

The patients had a baseline MRI which was designated as either normal or abnormal. *Abnormal baseline MRI* was defined by the presence of cerebral infarcts and/or cerebral atrophy on MRI at the time that the patient had the first study. *Worsening of MRI* over time was demonstrated by the presences of *new infarcts* and/or *new atrophy* or *progression of the baseline cerebral atrophy* on MRI by the time that the patient underwent the follow-up scan. The analysis of the MRI data was done firstly as ordinal data following an increasing order of MRI severity (0= better [or improved- not applicable for this variable]; 1= unchanged; and 2= worse), and secondly as binary data in relation to an outcome (0= better/unchanged; and 1= worse).

Baseline MRA was either normal or abnormal. *Abnormal baseline MRA* data were analysed as either ordinal or binary. Ordinal MRA data were defined by the presence of any grade of flow turbulence seen on the scan (coded in increasing grade of severity: 0= no turbulence; 1= mild; 2= moderate; 3= severe turbulence; 4= arterial occlusion; and 5= arterial occlusion plus collaterals [moyamoya syndrome]). An *improvement* on MRA over time was defined as a decrease in the grade of the flow turbulence or return to a normal pattern of blood flow signal. On the other hand, *worsening* in the MRA pattern of turbulence was defined by an increase on blood flow turbulence on MRA secondary to different grades of artery stenosis, or lack of MRA signal in the case of artery occlusion. As binary data, MRA turbulence was analysed in relation to an outcome (0= better/ unchanged [as a single category]; 1= worse).

The patients had either a normal or abnormal baseline transcranial Doppler ultrasound (TCD). *Abnormal transcranial Doppler ultrasound* was defined as the presence of an abnormal pattern of arterial blood flow velocities (middle and anterior cerebral arteries) following criteria modified from Adams (Adams et al 1992, Kirkham et al 2001). In these criteria the abnormal TCD patterns were classified as binary data (0= better/unchanged; 1=worse) or as ordinal data in increasing order of severity (0=normal; 1= middle cerebral artery (MCA) flow velocities more than 170 cm/sec and lower than 200 cm/sec; 2= MCA velocities equal to or higher than 200 cm/sec; 3= MCA velocities lower than 70 cm/sec and a ratio lowest/highest of ipsilateral MCA velocities equal or

lower than 0.5 or an anterior cerebral artery/MCA ipsilateral highest velocities more than 1.2 [evidence of artery stenosis]; 4= inability to record an MCA signal in the presence of a demonstrated ultrasound window). Pulsatility index was not considered for analysis (Adams et al 1992). An *improvement* of TCD was defined by a decrease in TCD category returning to a normal ultrasound pattern of blood flow or with evidence of better arterial blood flow. A *worsening* in TCD was defined as a progression of the abnormal ultrasound pattern of blood flow longitudinally.

Baseline perfusion MRI of the patients was defined as normal or abnormal. *Abnormal perfusion MRI* (or DSC-MRI) was characterised as the presence of a region of increased mean transit time (MTT) of the passage of intravenous Gadolinium bolus through the cerebral vasculature, or an increase or decrease of regional cerebral blood flow (CBF) or cerebral blood volume (CBV). An increase of MTT and/or decreased CBF or CBV is seen when there is cerebrovascular disease with artery stenosis or occlusion (Kirkham et al 2001) which leads to slow the passage of blood flow secondary to the arterial obstruction. An increase in CBF and/or CBV is related to hyperaemia due to adaptative vasodilatation secondary to anaemia (Prohnovik et al 1989). For statistical analysis, perfusion MRI data were used as binary data (0= unchanged/better; 1= worse), or the perfusion MRI parameters were used as ordinal data in increasing order of perfusion severity. Abnormal perfusion MRI was characterised by visual inspection of the relevant parameter map; e.g. a regional *increase in MTT* was defined with a *sign* '+' (=: normal ; +: mild ; + +: moderate; + + +: severe increase in MTT); a regional *decrease in CBF and/ or CBV* was defined with a *sign* '-' (=: normal; -: mild decrease; - - : moderate decrease; - - - : severe decrease in CBF or CBV). An *improvement* in cerebral perfusion on perfusion MRI was defined as a change in category of two steps or more towards normal for MTT, CBF or CBV. Conversely, a *worsening* in perfusion MRI was defined as a change of two or more categories away from normal. These criteria were adopted in order to be conservative in the identification of changes determined on visual assessment of parameter maps.

Statistical analysis was done using SPSS 10 for Windows. For the study of association among recurrent neurological symptoms, conventional neuroimaging (MRI and MRA), TCD and perfusion MRI, χ^2 and Fisher's exact test were used for binary categorical

data, the Mann-Whitney test for associations between binary data and ordinal data, and Spearman's test for ordinal data. For clinical data (follow-up blood pressure and oxygen saturations) logistic regression was used to investigate association between continuous data and binary categorical data in relation to an outcome (presence of recurrent neurological symptoms or not); T-Test was used to compare parametric continuous data (initial haematological parameters), and Mann-Whitney test for non-parametric continuous data (follow-up haematological parameters) against an outcome (worsening of perfusion MRI, MRA and MRI). Level of significance was set at $p<0.05$ and a trend for significance was defined as $p \geq 0.05$ and $p \leq 0.1$. In relation to the multiple comparisons issue, it is necessary to examine the level of dependence among the set of explanatory variables since, given a set of associated variables, the tests are not independent. The correlation matrix has been used for this purpose. Although correlation matrix calculations might usually be reserved for interval or ratio data, correlation matrix calculations (multivariate analyses) based on ordinal data is accepted in social science data analysis (Afifi and Lark, 1990). The correlation matrix provides an immediate visual impression of the association among the set of explanatory variables and avoids the complication that arises when multidimentional contingency tables are used for this purpose.

5.4. Results I- Neurological Symptoms and Clinical Parameters

5.4.1. Patients and Clinical Symptoms

5.4.1.1. Clinical Symptoms- Central Nervous System Events at Presentation

The clinical symptoms of the patients are described for the 23 patients included in the study, i.e. those who had a successful second perfusion MRI study (figure 5.1). The main neurological syndrome at presentation was described in every patient with the clinical data obtained to date, and other recurrent symptoms (such as headaches, seizures and learning difficulties) considered as secondary features if they were not the main neurological symptom for a given patient. All patients had neurological symptoms initially (figure 5.1). As the main symptom at presentation, 3 patients had coma, 7 patients had overt infarction (clinical stroke, 29%); 1 stroke patient had accompanying

symptoms with decreased visual acuity secondary to a parietal-occipital lobe infarction. Six patients had transient ischaemic attacks (TIA, 26%), of whom 4 presented with anterior territory TIA, and 2 with posterior territory TIA. One patient had seizures, and 6 headaches (26%).

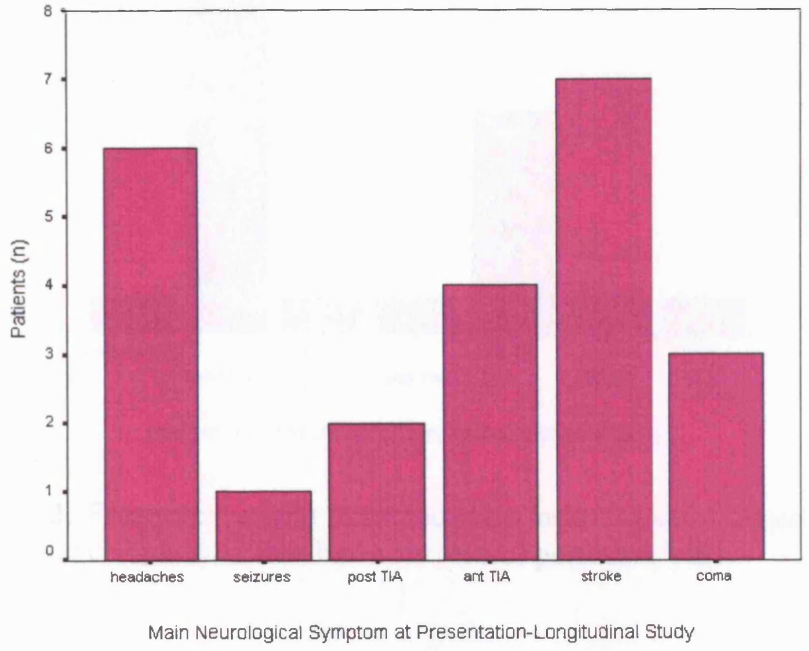


Figure 5.1. Frequency of different central nervous system events at presentation of sickle cell patients who had a successful second perfusion MRI.

5.4.1.2. Clinical Symptoms- Recurrent Neurological Symptoms During Follow-up

During follow-up, the main recurrent neurological symptom, in terms of severity, was considered for analysis (figure 5.2). One patient presented with coma (posterior leukoencephalopathy-PLKE-[coma and hypertension]; 1 patient re-stroked; 7 patients had TIAs (30%) (6 had anterior territory TIAs [26%] and 1 had a posterior territory TIA); 1 patient had seizures; and 12 had recurrent headaches (52%); one patient maintained asymptomatic.

However, other recurrent symptoms (on one or more occasions) were manifested in the patients in addition to the main recurrent neurological symptom, such as TIAs in one;

seizures in 2; headaches in 5; and cognitive symptoms (learning difficulty and behaviour problems) in 14 patients.

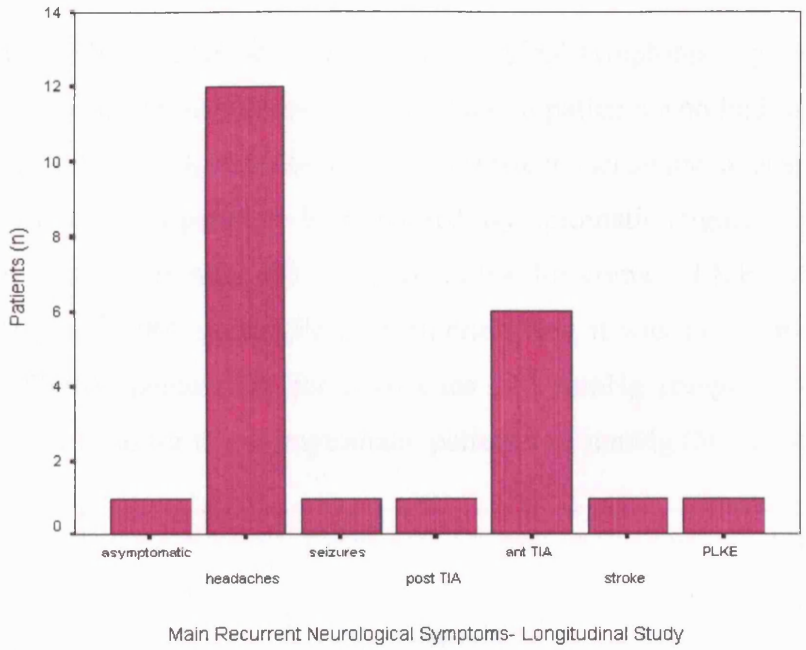


Figure 5.2. Frequency of the main recurrent neurological symptoms of the sickle cell patients who had a successful perfusion MRI.

Most of these patients were on therapy. Eighteen out of 23 patients were on chronic blood transfusion therapy. However, during follow-up, 7/18 patients stopped blood transfusion: 1 patient due to antibody formation against transfused red cells, 1 patient because she received a bone marrow transplant, 1 patient due to poor compliance with blood transfusion therapy, 1 patient because a decision was made that further therapy was unnecessary, and 3 patients who continued treatment on Hydroxyurea (1 of these 3 patients also had a left frontal revascularisation procedure). In addition, 1 patient was on Hydroxyurea (without having previous blood transfusion), and 4 patients had adenotonsillectomy for obstructive sleep apnoea.

5.4.2. Blood Pressure Measurement and Haematological Parameters

5.4.2.1. Blood Pressure Measurements

5.4.2.1.1. Systolic Blood Pressure

Systolic blood pressure (SBP) was measured in 15 out 23 patients at follow-up.

At the final scan, the mean SBP of the patients was 113 mmHg (range 97 to 128 mmHg).

In relation to the effect of SBP on recurrent neurological symptoms (figure 5.3), follow-up systolic blood pressure measurements were done in patients who had recurrent stroke (n=1), coma -PLKE- (n=1), anterior territory transient ischaemic attack -TIA- (n=6), headaches (n=6), and in a patient who remained asymptomatic (figure 5.3). For stroke, the mean SBP was 112 mmHg (50-75th percentile), for coma [PLKE], the mean SBP was 117 mmHg (75th –90th percentile), for anterior TIA, it was 112 mmHg (range 97-128 mmHg; <50-90th percentile), for headaches 113 mmHg (range 104-125 mmHg; <50-90th percentile) and for the asymptomatic patient 115 mmHg (50th percentile).

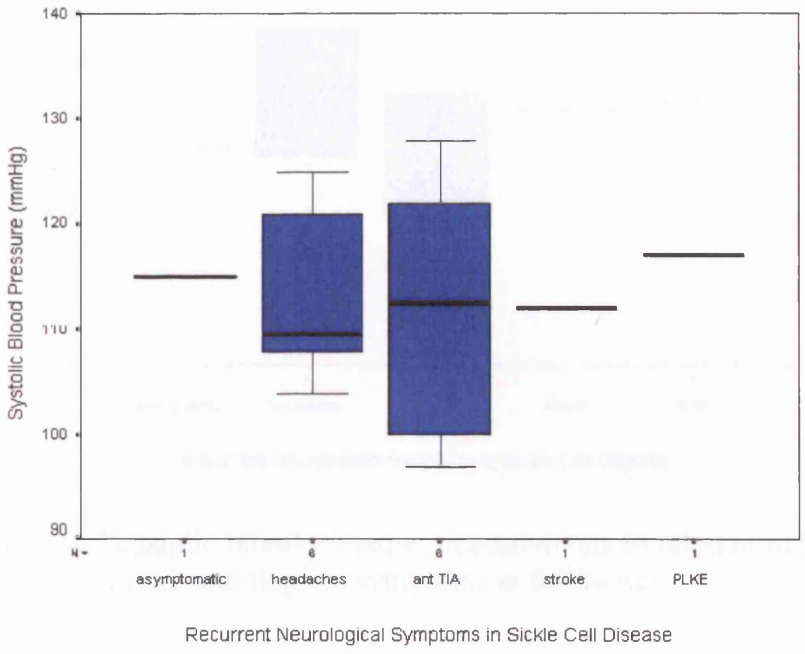


Figure 5.3. Systolic blood pressure measurements in relation to SCD patients' recurrent neurological symptoms at follow-up.

5.4.2.1.2. Diastolic Blood Pressure

Diastolic blood pressure (DBP) was measured in a total of 15 of the 23 patients.

At the final scan, the mean DBP of the sickle cell patients was 52 mmHg (range 30 to 79 mmHg).

In relation to the association of DBP with recurrent neurological symptoms (figure 5.4), follow-up diastolic blood pressure measurements were done in patients who had recurrent stroke (n=1), coma –PLKE- (n=1), anterior TIA (n=6), headaches (n=6) and in a patient who remained asymptomatic (figure 5.4). For stroke, the mean DBP was 54 mmHg (<50th percentile), for coma [PLKE] 55 mmHg (<50th percentile), for anterior TIAs 49 mmHg (range 30-75 mmHg; <50th to 75th percentile), for headaches was 56 mmHg (range 31-79 mmHg; <50-90th percentile) and for the asymptomatic patient was 49 mmHg (<50th percentile).

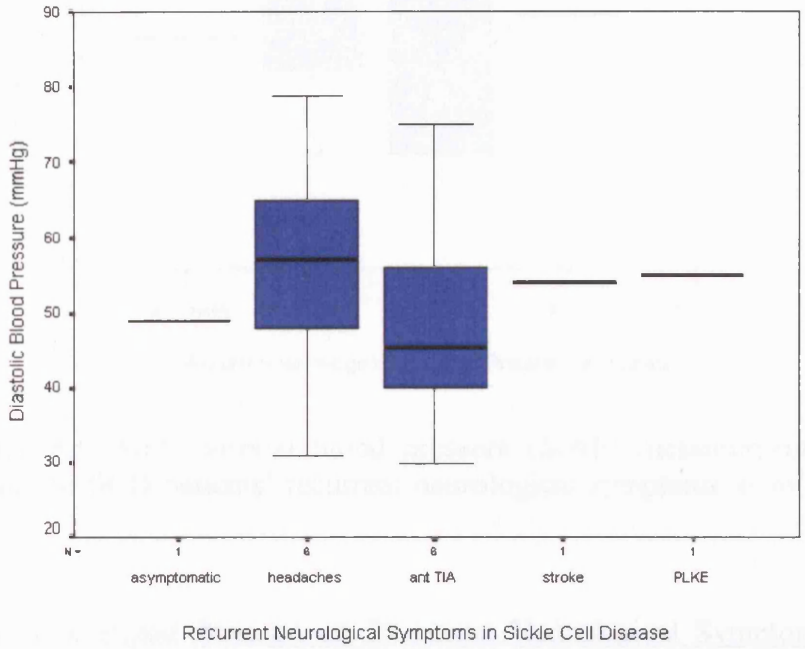


Figure 5.4. Diastolic blood pressure measurements in relation to SCD patients' recurrent neurological symptoms at follow-up.

5.4.2.1.3. Mean Arterial Blood Pressure (MAP)

Mean arterial blood pressure (MAP) was measured in 15 of the 23 patients.

At the final scan, the mean MAP of these patients was 74 mmHg (range 58 to 97 mmHg).

In relation to recurrent neurological symptoms (figure 5.5.), follow-up MAP measurements were done in patients who had recurrent stroke (n=1), coma –PLKE- (n=1), anterior TIA (n=6), headaches (n=6) and in a patient who remained

asymptomatic. For stroke, the mean MAP was 74 mmHg, for coma [PLKE] it was 76 mmHg, for anterior TIA 72 mmHg (range 58-90 mmHg), for headaches 77 mmHg (range 69-97 mmHg) and for the asymptomatic patient was 72 mmHg.

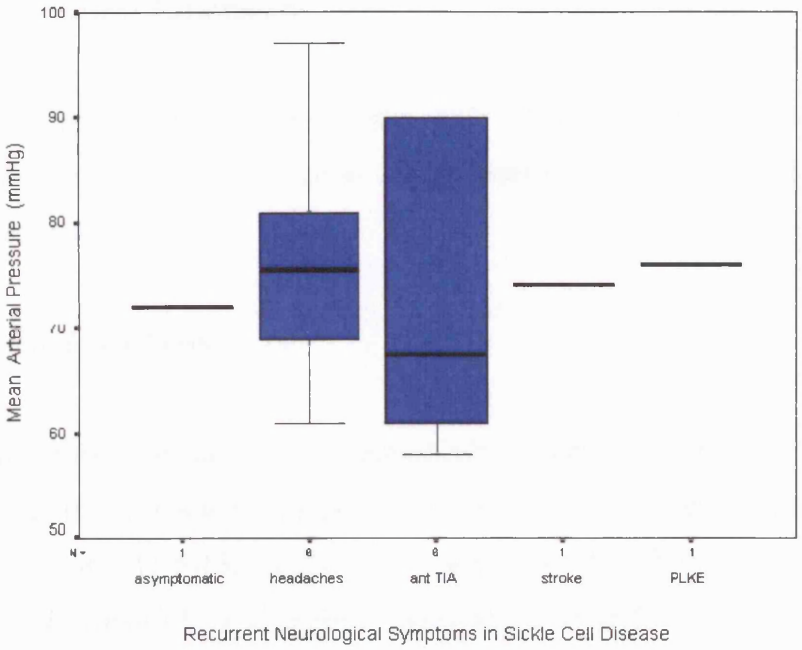


Figure 5.5. Mean arterial blood pressure (MAP) measurements in relation to SCD patients' recurrent neurological symptoms at follow-up.

5.4.2.1.4. Effect of Blood Pressure on Recurrent Neurological Symptoms and MRI, MRA, TCD , and Cerebral Perfusion Changes Longitudinally- Statistical Analysis

Follow-up mean systolic, diastolic and mean arterial blood pressures were not significantly associated with the presence of neurological symptoms in the sickle cell patients of this study (p=0.79, p= 0.79 and p=0.91 respectively, logistic regression). There were also no significant associations between follow-up mean SBP, DBP and MAP and worsening of MRI (p=0.48, p=0.83 and p=0.85 respectively), worsening of MRA (p=0.86, p=0.36 and p=0.5 respectively), and worsening of cerebral perfusion (p=0.63, p=0.75 and p=0.96 respectively) longitudinally. There was a trend for association between worsening of TCD and diastolic blood pressure (p=0.175), where those patients with worse TCD over time had lower DBP measurements (mean 44 [range 30-61] mmHg) than those with better or unchanged TCD (mean 56 [range 42-74]

mmHg); however there were no significant associations for SBP and MPA with TCD (p=0.67 and p=0.41 respectively).

5.4.2. Haematological Parameters

Haematological data of the patients of this study were collected from clinical records. For this study the nearest blood test at steady state to the neuroimaging at onset and follow-up were taken.

5.4.2.1. Haemoglobin Level

Haemoglobin (Hb) level at onset: Haemoglobin level data were collected from 15 patients. Mean Hb level was 9.10 g/dL, range from 6.5 to 13.2 g/dL. Normal values for age are: 6-12 years: 11.5-15.5 g/dL; 12-18 years (male): 13-16 g/dL, (female):12-16 g/dL; 18-28 years (male):13.5-17.5 g/dL, (female): 12-16 g/dL.

Haemoglobin (Hb) level at follow-up: Haemoglobin level data were collected from 12 patients. Mean Hb level was 8.9 g/dL, with a range from 6.1 to 10.9 g/dL.

5.4.2.2. Haemoglobin S%

Haemoglobin S% (HbS%) at onset: No data.

Haemoglobin S% (HbS%) at follow-up: Haemoglobin S% level data were collected from 5 patients. Mean HbS% level was 49.12%, with a range from 17 to 90.7%.

5.4.2.3. White Cell Count

White Cell Count (WCC) at onset: White cell count data were collected from 15 patients. Mean WCC was 11.8 x 10^9/L cells, with a range from 6.9 to 20.8 x 10^9/L cells. Normal values for group age are: 4-7 years: 5.5-15.5 x 10^9/L cells; 8-13 years: 4.5-13.5 x 10^9/L cells; 14-28 years: 4.5 11.0 x 10^9/L cells.

White Cell Count (WCC) at follow-up: White cell count data were collected from 12 patients. Mean WCC was 13.2 x 10^9/L cells, with a range from 7.5 to 50 x 10^9/L cells.

5.4.2.4. Neutrophils

Neutrophils at onset: No data.

Neutrophil at follow-up: Neutrophil count data were collected from 9 patients. Mean neutrophil count was 11.82 x 10^9/L cells, with a range from 1.9 to 43.8 x 10^9/L cells (normal neutrophil 'segs' 3.0 -5.8 cells x 10^9/L).

5.4.2.5. Lymphocytes

Lymphocytes at onset: No data.

Lymphocytes at follow-up: Lymphocyte count data were collected from 9 patients. Mean lymphocyte count was 3.86 x 10^9/L cells, with a range from 1.7 to 5.7 x 10^9/L cells (normal 1.5-3.0 x 10^9/L cells).

5.4.2.6. Platelets

Platelets at onset: Platelet counts were collected from 15 patients. Mean platelet count was 311 x 10^9/L cells, with a range from 112 to 504 x 10^9/L cells (normal 150-400 x 10^9/L cells).

Platelets at follow-up: Platelet count data were collected from 12 patients. Mean platelet count was 374 x 10^9/L cells, with a range from 239 to 594 x 10^9/L cells (normal 150-400 x 10^9/L cells).

5.4.2.11. Relation of Haematological Parameters at Onset and Follow-up with Worsening Perfusion, MRI and MRA- Statistical Analysis

Worsening perfusion MRI was not significantly associated with initial haemoglobin level (p=0.94, T-Test), white cell count (p=0.84) and platelet count (p=0.22). However,

there was a trend for association between worsening of cerebral perfusion over time and increased white cell count at follow-up (p=0.089, Mann-Whitney test, figure 5.6), but worsening of cerebral perfusion was not significantly associated with follow-up Hb levels (p=0.2), neutrophils (p=0.7), lymphocytes (p=0.8) or platelets (p=0.73)

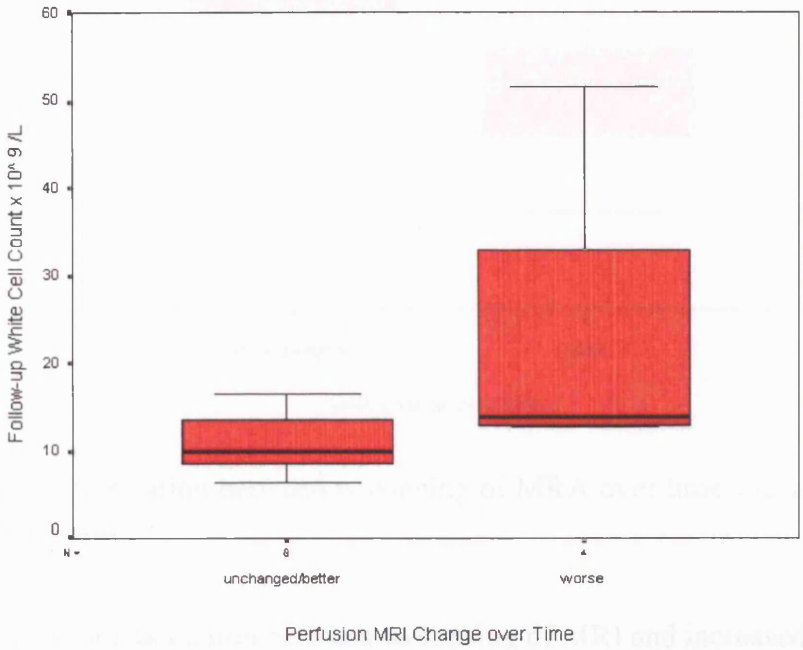


Figure 5.6. Relation between worsening of perfusion MRI over time and white cell count at follow-up.

Worsening of MRA was not associated with initial Hb level (p=0.39, T-test), on white cell count (p=0.4), but it was significantly associated with decreased initial platelet count (p=0.008, figure 5.7). In addition, worsening of MRA was not associated with follow-up Hb levels (p=0.47, Mann-Whitney test), white cell count (p=0.34), neutrophils (p=0.52), lymphocytes (p=0.44) or platelet count (p=0.94).

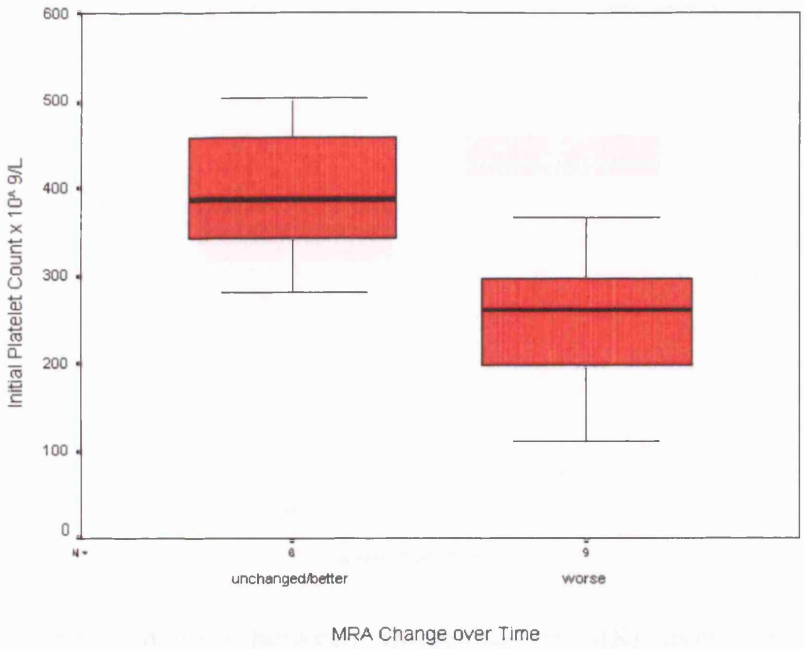


Figure 5.7. Relation between worsening of MRA over time and initial platelet count.

There were trends for association between worsening of MRI and increased follow-up white cell count (p= 0.12, Mann-Whitney test, figure 5.8) and lymphocytes (p=0.14, figure 5.9). There were no significant associations for initial Hb level, white cell or platelets counts (p=0.9, p=0.46 and p=0.48, respectively, T-test) or for follow-up Hb level, neutrophil or platelet counts (p=0.63, p=0.71 and p=0.8, Mann-Whitney test).

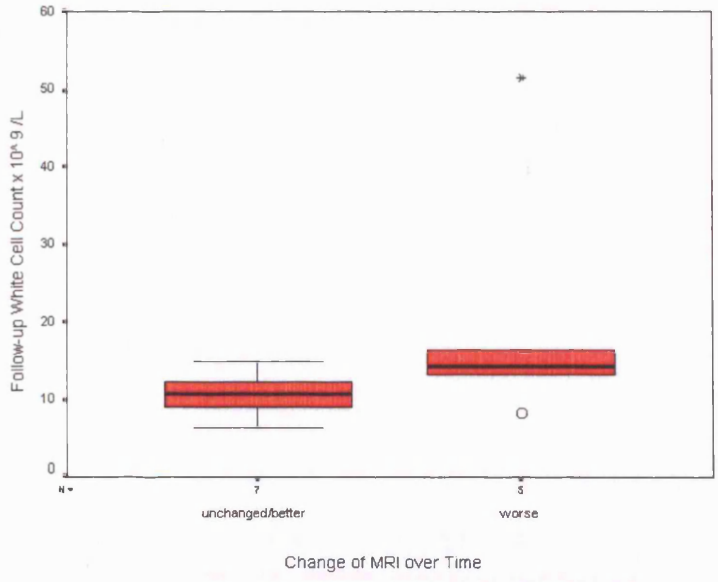


Figure 5.8. Relation between worsening of MRI over time and white cell count at follow-up.

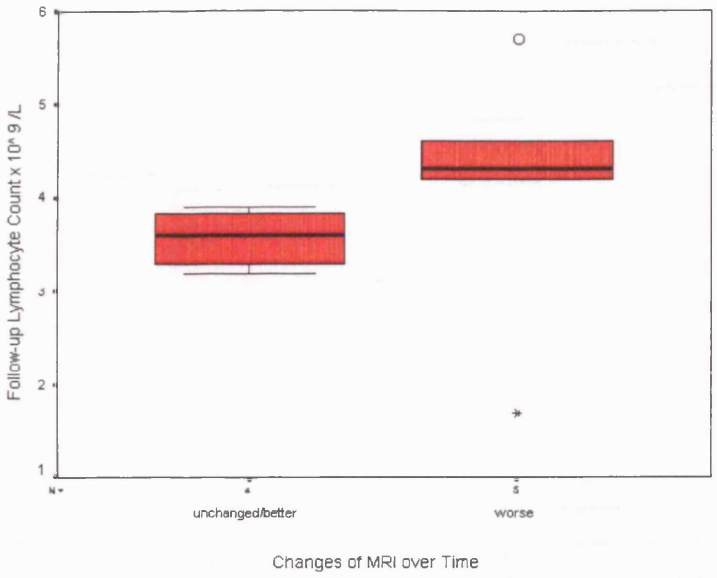


Figure 5.9. Relation between worsening of MRI over time and lymphocyte count at follow-up.

5.4.3. Awake Oxygen Saturation

Blood oxygen saturation (SpO_2) was measured with a pulse oximeter (chapter 2) for three minutes in 20 out of 23 sickle cell patients, just before or after the MR studies at the final MR scans. Median SpO_2 was 96%, range 92 to 98.4%. As described in chapter 2, the cut-off used for hypoxaemia is $SpO_2 < 92$, therefore these patients had day time oxygen saturations within the normal limits accepted by the GOSH Respiratory Laboratory (figure 5.10).

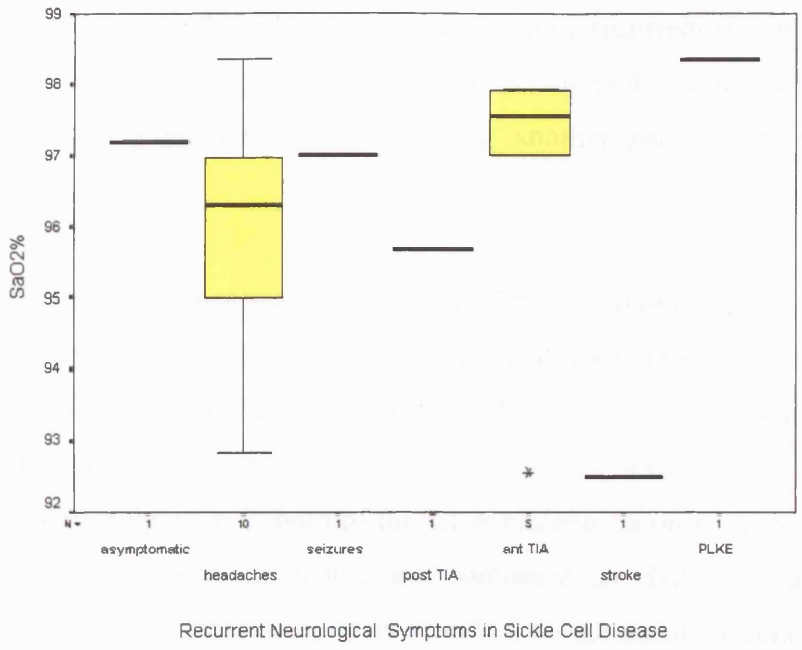


Figure 5.10. Awake Oxygen Saturation in Relation to SCD Patients' Recurrent Neurological Symptoms.

5.4.3.1. Effect of Awake Oxygen Saturations on Recurrent Neurological Symptoms and MRI, MRA, TCD, and Cerebral Perfusion Changes Longitudinally – Statistical Analysis

Follow-up awake oxygen saturations (SpO_2) of these patients were not significantly associated with the presence of recurrent neurological symptoms (p=0.58, logistic regression). There was also no significant association between awake SpO_2 and worsening of MRI (p=0.68), worsening of MRA (p=0.94), worsening of TCD (p=0.39) or worsening of cerebral perfusion (p=0.96).

5.5. Results II: Recurrent Neurological Symptoms and MR and TCD Investigations

5.5.1. Association between MRI and Recurrent Neurological Symptoms Longitudinally

Twenty three sickle cell patients underwent initial and follow-up T2- weighted MRI scans. Seventeen patients had unchanged MRI and 6 patients had worsening of MRI during a follow-up of a mean of 2.2 years (range 8 months to 3.5 years, Appendix tables 3 and 4).

Of the patients who had longitudinally unchanged MRI, 9/17 had normal MRI throughout; all of these patients had headaches as the main recurrent symptom; 2/9 were on chronic blood transfusion therapy, which was stopped during the follow-up, although, one patient continued on Hydroxyurea; another patient was treated with Hydroxyurea only.

Eight of 17 patients had unchanged MRI with an abnormal baseline MRI. Five out of eight patients had anterior territory transient ischaemic attacks (TIAs); all of these sickle cell patients were on blood transfusion therapy but 3 of them stopped treatment during follow-up. MRI abnormalities consisted of small covert infarcts (unilateral in 1, multiple and bilateral infarcts in 2; both of the latter patients stopped blood transfusion, but one had a revascularisation procedure and continued on Hydroxyurea); unilateral multiple covert and overt infarcts in a patient who had stroke despite blood transfusion; and multiple covert and overt infarcts in an another patient who also had stroke but stopped blood transfusion and received a bone marrow transplant. Two out of eight patients with abnormal baseline and unchanged MRI presented with headaches; both patients were on blood transfusion and had a history of stroke, one had a medium sized unilateral infarct and the other had bilateral large infarcts and cerebral atrophy. One out of 8 patients, who had a history of stroke and continued with chronic blood transfusion, had unchanged MRI and no recurrent symptoms.

Six out of 23 patients had worsening of MRI during the follow-up. All of them were on blood transfusion therapy initially, but two patients stopped treatment. Five of these 6 patients had had previous stroke, with overt infarction (4 bilateral, 1 unilateral), and 1 patient had had a posterior territory TIA and a normal baseline MRI.

During follow-up, 3 patients (2 with history of stroke) had new infarcts (2 unilateral and 1 bilateral) and 3 other patients had new cerebral atrophy. The recurrent neurological symptoms in the patients who had history of stroke were the following: coma -posterior leukoencephalopathy- (n=1; *new unilateral infarct*), re-stroked (n=1; *new unilateral infarct*; stopped blood transfusion due to antibody formation against transfused red cells), anterior territory TIA (n=1; *new atrophy*), seizures (n=1; *new cerebral atrophy*), and headaches in conjunction with decreased visual acuity (n=1; *new occipital cerebral atrophy*). One patient without history of stroke and worsening of MRI had further

posterior territory TIAs on discontinuing blood transfusion (n=1, *new bilateral watershed infarcts).*

Only one patient with a normal baseline MRI developed new infarction (covert) and transient neurological symptoms (posterior territory TIA [table 5.1]). Five out of 6 patients with worsening MRI scans had had previously abnormal baseline MRIs and all were on blood transfusion. Only one patient discontinued treatment and re-stroked (table 5.1). Patients (n=9) with normal baseline, which persisted normal through the study, had less severe symptoms such as headaches. However, those patients with abnormal baseline and unchanged MRI (all of them initially on blood transfusion) presented with a variety of recurrent symptom severity from being asymptomatic (only one patient) to TIAs (predominantly). The patients (n=2), who had the most severe recurrent symptoms (stroke and coma [PLKE]) had abnormal baseline and worsening MRI despite being on chronic blood transfusion, although one of them stopped blood transfusion because of antibody formation against the transfused red cells. The effect of blood transfusion therapy on MRI changes over time will be discussed in chapter 6.

Recurrent Neurological Symptoms (n=patients)	Change Over Time (n= 23 Sickle Cell Patients) MRI					
	Better (BTx)		Unchanged (BTx)		Worse (BTx)	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
Coma (PLKE) (n=1)						1 (1) *F*
Stroke (n=1)						1 (1) [1§] *G*
Anterior Territory TIA (n=6)				5 (5) [1§] [1§HU+RV] [1§BMT] *B*		1 (1) *H*
Posterior Territory TIA (n=1)					1 (1) [1§] *E*	
Seizures (n=1)						1 (1) *I*
Headaches (n=12)			9 (3) [2§] [1 HU] [1§HU] *A*	2 (2) *C*		1 (1) *J*
Asymptomatic (n=1)				1 (1) *D*		

Table 5.1. Changes over time on MRI in relation to recurrent neurological symptoms and therapy in patients with sickle cell disease. Longitudinal study.

BTx: Blood transfusion therapy

PLKE: Posterior leukoencephalopathy

TIA: Transient ischaemic attack

[HU] Number of sickle cell patients on Hydroxyurea therapy

[§] Number of patients who stopped blood transfusion therapy during follow-up

[§HU] Number of patients who stopped blood transfusion therapy and continued with Hydroxyurea during follow-up

[§BMT] Number of patients who stopped blood transfusion therapy and had bone marrow transplant during follow-up

[§HU+RV] Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up.

No data because no improvement can be demonstrated from a normal study or from a study showing the presence of lesions.

Patients from Appendix Tables 3 and 4 (Patient number): ***A:*** 3, 15, 17,18, 19, 20, 21, 22, 23 / ***B:*** 7,10, 12, 13, 14 / ***C:*** 2, 5 / ***D:*** 11/ ***E:*** 16 / ***F:*** 1/ ***G:*** 8/ ***H:*** 6/ ***I:*** 4/ ***J:*** 9

5.5.1.1. MRI Predictors of worsening MRI and Recurrent Neurological Symptoms over Time

Previous stroke (overt cerebral infraction) was the main predictor of worsening of MRI and recurrent neurological symptoms such as coma (PLKE), stroke recurrence and seizures. These patients were initially on chronic blood transfusion therapy, which did not prevent stroke recurrence in 1/3 patients, or progression to cerebral atrophy in a further 2. Only one patient with previous history of stroke and on blood transfusion remained asymptomatic and with unchanged follow-up MRI.

Patients who had recurrent anterior territory TIAs were initially on blood transfusion and all had infarction on the baseline MRIs. Three out of 5 patients stopped transfusion and 2 of those continued with alternative treatments (table 5.2).

Patients with normal baseline MRI had mainly headaches and remained with normal MRI studies throughout, except for one patient who had a posterior territory TIA and had recurrent posterior TIA and covert infarcts on the follow-up MRI (having stopped blood transfusion).

5.5.1.2. Changes Over Time on MRI and Recurrent Neurological Symptoms in SCD-Statistical Analysis

The manifestation of recurrent neurological symptoms was not significantly associated with worsening of MRI (p=1, Fisher's exact test). Excluding headache, which has not been clearly demonstrated to be a symptom of vascular insufficiency (Pavlakis et al 1986), symptoms such as stroke, TIAs and seizures, were selected for having been reported as manifestation of ischaemia or/and cerebrovascular disease in SCD (Powars 1978, Pavlakis et al 1989, Ohene-Frempong et al 1991, Liu et al 1994, Miller et al 1999, Prengler et al 2002). Analysis of the data showed a weak trend for patients who presented with stroke, seizure or TIAs to have worsening of MRI (p=0.16; Fisher's

exact test). Recurrent headaches and anterior TIA were significantly associated with unchanged MRI (p=0.002 and p=0,009; Fisher's exact test). It was not possible to analyse associations between other symptoms and unchanged MRI, and between individual recurrent symptoms and worsening of MRI because the numbers were too small.

Analysing the whole group of recurrent neurological symptoms as ordinal data with increasing impairment, the severity of the recurrent symptoms was significantly associated with changes on MRI over time and infarct number (p=0.029 and p=0.001 respectively; Spearman's correlations, tables 5.2 and 5.3). Patients without or with less severe symptoms had unchanged MRI, but patients with anterior territory TIAs also had unchanged MRI. Patients who re-stroked and the one presenting with coma (PLKE) had a worse MRI at follow-up.

	Changes on MRI		**Total**
Recurrent Symptom	**Unchanged**	**Worse**	
Asymptomatic	1		1
Headache	11	1	12
Seizures		1	1
Posterior TIA		1	1
Anterior TIA	5	1	6
Stroke		1	1
Coma (PLKE)		1	1
Total	17	6	23

Table 5.2. Severity of recurrent neurological symptoms in sickle cell patients and changes on MRI over time.

Patients with 2 or more infarcts at follow-up (new or old infarcts) were more likely to have severe recurrent neurological symptoms such as seizures, TIAS, stroke or coma (PLKE) (table 5.3).

Recurrent Symptom	Infarct Number at Follow-Up					Total
	No Infarct	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
Asymptomatic					1	1
Headache	9	1	2			12
Seizures					1	1
Posterior TIA					1	1
Anterior TIA			2	1	3	6
Stroke					1	1
Coma (PLKE)					1	1
Total	9	1	4	1	8	23

Table 5.3. Recurrent neurological symptoms in sickle cell patients and infarct **number** on **follow-up** MRI.

There was a trend for an association between the severity of the recurrent neurological symptoms and infarct size (p=0.059, Spearman's correlation, table 5.4). Patients with moderate to large cerebral infarcts on MRI at follow-up had recurrent seizures or stroke or in a fewer number TIAs. However, those with small infarcts also have predominantly TIAs (anterior and posterior territories) and coma (PLKE).

Recurrent Symptom	Infarct Size				Total
	No infarct	< 1cm-small	1-5 cm-moderate	> 5 cm-large	
Asymptomatic			1		1
Headache	9		2	1	12
Seizures			1		1
Posterior TIA		1			1
Anterior TIA		4	1	1	6
Stroke				1	1
Coma (PLKE)		1			1
Total	9	6	5	3	23

Table 5.4. Recurrent neurological symptoms in sickle cell patients and infarct **size** on **follow-up** MRI.

5.5.2. Association of MRA and Recurrent Neurological Symptoms

Twenty three patients underwent initial and follow-up MR angiogram (MRA). Four out of 23 patients had an improvement in the grade of MRA turbulence, 10 patients had unchanged studies, and 9 patients had a worsening of the MRA turbulence on the final

scans (table 5.5). The categorisation of MRA abnormality in terms of on flow turbulence/occlusion, was based on the most severe grade of MRA abnormality for each patient.

All four patients with an improved MRA had an abnormal baseline study, and all of them were initially treated with blood transfusion, although 3 patients stopped this treatment during follow-up. However, these patients manifested a variety of recurrent neurological symptoms (patients' treatments in table 5.5). In this group of patients, improvement in MRA turbulence was seen in the anterior cerebral circulation (terminal internal carotid artery [TICA], middle cerebral artery [MCA] and anterior cerebral artery [ACA]). MRA improved from moderately severe (grade 2-3) to normal (grade 0) or mild (grade 1) turbulence. One patient had an anterior territory TIA, and another presented with a recurrent posterior territory TIA. The remaining two patients had headaches; in one the MRA improved to normal in association with blood transfusion, but tICA turbulence returned when he stopped treatment (patient no. 20).

Ten patients had unchanged MRA throughout the study; 7 of the 10 had a normal baseline MRA, and 3 had an abnormal baseline study. Six of the 7 patients with normal baseline MRA (which remained normal during follow-up) had predominantly headaches. Only one patient presented with coma and hypertension (PLKE; he had a previous history of stroke). Three patients had abnormal baseline MRA turbulence in the anterior circulation. These patients had a range of recurrent neurological symptoms, one patient with artery occlusion had recurrent seizures; one patient with moderate artery turbulence continued to have headaches; the remaining patient with mild artery turbulence had no recurrent symptoms during follow-up.

Nine patients had worsening of MRA at the final scan. One of the 9 patients had a baseline normal MRA with progression from a normal study to mild turbulence in the anterior circulation, and he had headaches. Eight patients had an abnormal baseline MRA, all of who were initially on a blood transfusion programme. One patient re-stroked with progression from unilateral to bilateral artery occlusion, collaterals and severe turbulence in other vessels. Four out of the remaining 7 patients had anterior territory TIAs with a baseline MRA with moderate to severe turbulence, which progressed to artery occlusion and collaterals (n=1), collaterals (n=1), or severe

turbulence with pre-existent collaterals (n=2). In one, new turbulent flow developed in previously normal vessels (n=1). Two of 8 patients had headaches; MRA changed from severe turbulence to occlusion and moyamoya syndrome in one; and from moyamoya syndrome to the addition of artery turbulence in the posterior vascular territory in the other. These two patients had stroke and they were on chronic blood transfusion.

As can be seen in table 5.5, only one patient with a normal baseline MRA had worse MRA at follow-up. Patients with stroke and anterior TIA had a baseline MRA that was worse at follow-up, except for one patient. Patients with headaches had predominantly normal baseline MRA which remained unchanged.

Change Over Time (n=23 Sickle Cell Patients) MRA						
Recurrent Neurological Symptoms (n=patients)	**Better (BTx)**		**Unchanged (BTx)**		**Worse (BTx)**	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
Coma (PLKE) (n=1)			**1 (1)** *D*			
Stroke (n=1)						**1 (1)** [1 §] *J*
Anterior Territory TIA (n=6)		**1 (1)** [1 §] *A*				**5 (5)** 1§BMT 1§HU+RV *K*
Posterior Territory TIA (n=1)		**1 (1)** [1 §] *B*				
Seizures (n=1)				**1 (1)** *F*		
Headaches (n=12)		**2 (1)** [1 §] *C*	**6 (2)** [1 §] 1 §HU 1 HU *E*	**1 (1)** *G*	**1** *I*	**2 (2)** *L*
Asymptomatic (n=1)				**1 (1)** *H*		

Table 5.5. Changes over time on MRA in relation to recurrent neurological symptoms and therapy in patients with sickle cell disease.

BTx: Blood transfusion therapy
PLKE: Posterior leukoencephalopathy
TIA: Transient ischaemic attack
[HU] Number of sickle cell patients on Hydroxyurea therapy
[§] Number of patients who stopped blood transfusion therapy during follow-up
[§HU] Number of patients who stopped blood transfusion therapy and continued with Hydroxyurea during follow-up
[§BMT] Number of patients who stopped blood transfusion therapy and had bone marrow transplant during follow-up
[§HU+RV] Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up
■ No data because no improvement can be demonstrated from a normal MRA

Patients from Appendix Tables 3 and 4 (Patient number): ***A:*** 12 / ***B:*** 16/ ***C:*** 18, 20/ ***D:*** 1/ ***E:*** 3, 15, 17, 19, 21, 22 / ***F:*** 4/ ***G:*** 2/ ***H:*** 11/ ***I:*** 23/***J:*** 8 / ***K:*** 6, 7, 10, 13, 14/ ***L:*** 5, 9

5.5.2.1. Predictors of Recurrent Neurological Symptoms and Worsening MRA

Sickle cell patients who manifested the most severe recurrent neurological symptoms, such as stroke and anterior territory TIAs, had a worsening MRA pattern, as an indicator of progression of cerebrovascular disease (CVD), despite being on blood transfusion therapy. Patients with seizures, headaches or asymptomatic had unchanged or improved MRA pattern throughout; however, seizures could accompany other more severe neurological symptoms such as TIA (not included here, because the most severe recurrent neurological symptom was used in the analysis for each patient), and might also be a symptom of progression of CVD (see Chapter 7). The two patients who had recurrent headaches associated with worsening on MRA were patients with a previous history of stroke and CVD.

5.5.2.2. Changes over Time on MRA and Recurrent Neurological Symptoms in SCD-Statistical Analysis

Abnormal MRA at follow-up was not significantly associated with the presence of recurrent neurological symptoms, including headaches (p=1, Fisher's exact test). However, there were trends for associations between worse MRA and recurrent symptoms of stroke, TIAs or seizures (p= 0.07, Fisher's exact test) and recurrent TIAs (p=0.06, Fisher's test). There was also a trend for association between recurrent headaches and unchanged MRA at follow-up (p= 0.06, Fisher's test); other symptoms could not be analysed independently because of the small numbers.

Severity of grade of MRA turbulence at follow-up was significantly associated with recurrent stroke, TIAs or seizures (p=0.007, Mann-Whitney's test, table 5.6).

Recurrent Symptom TIA, Stroke, or Seizures	**MRA Turbulence at Follow-up**						**Total**
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
Absence	8	2	2			2	14
Presence		1	2	2	2	2	9
Total	8	3	4	2	2	4	23

Table 5.6. Relation between grade of MRA turbulence at follow-up and recurrence of stroke, transient ischaemic attack TIA or seizures (p=0.007).

Increasing grade of MRA turbulence at follow-up was significantly associated with MRA changes over time (better, unchanged and worse), and there was a trend for an association between the severity of recurrent neurological symptoms and increase in MRA turbulence (p= 0.002 and p=0.05 respectively, Spearman's correlation, tables 5.7 and 5.8).

Changes on MRA over Time	**MRA Turbulence at Follow-up**						**Total**
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + collaterals	
Better	1	1	2				4
Unchanged	7	1	1		1		10
Worse		1	1	2	1	4	9
Total	8	3	4	2	2	4	23

Table 5.7. Relation between grade of MRA turbulence at follow-up and MRA change over time.

Recurrent Symptom	**MRA Turbulence at Follow-Up**						**Total**
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collaterals	
Asymptomatic		1					1
Headache	7	1	2			2	12
Seizures					1		1
Posterior TIA		1					1
Anterior TIA			2	2	1	1	6
Stroke						1	1
Coma (PLKE)	1						1
Total	8	3	4	2	2	4	23

Table 5.8. Relation between grade of MRA turbulence at follow-up and recurrent neurological symptoms.

5.5.3. Association of Transcranial Doppler Ultrasound and Recurrent Neurological Symptoms

Seventeen sickle cell patients underwent initial and follow-up transcranial Doppler ultrasound (TCD, which assessed mainly cerebral anterior circulation (MCA, ACA). The patient who re-stroked and the one who had coma (posterior leukoencephalopathy)

as recurrent neurological symptoms did not have initial TCD and they were not included in this section. Median maximum MCA velocities (maximum velocity of both MCAs) were 115 cm/sec (range 30- 219 cm/sec-available data-) at onset, and 91 cm/sec (range 0-220 cm/sec) at follow-up.

Two of 17 patients had improved TCD, 8 had unchanged TCD, and 7 had worsening TCD at the end of the study (see table 5.9).

The two patients with improved TCDs had abnormal baseline studies. One patient had an anterior territory TIA, stopped blood transfusion, had a revascularisation procedure and continued with Hydroxyurea. This patient had initially an undetectable MCA, and at follow-up, a detectable MCA with decreased velocities less than 70 cm/sec (lowest: highest ratio ≤ 0.5). The other patient (who had headaches) had at onset decreased MCA velocities less than 70 cm/sec, and had normal TCD as her final study.

Eight patients had unchanged TCD. Two of the 8 patients continued to have normal studies and had recurrent headaches. Six of 8 patients had abnormal baseline studies, all of them were initially on blood transfusion: 2 patients had anterior territory TIAs and undetectable MCA (n=1), and low MCA flow velocities < 70 cm/sec (n=1). One patient had seizures (who had stroke and MCA velocities > 200 cm/sec in her first TCD before being included in this study), and two patients with headaches maintained MCA velocities < 70 cm/sec throughout.

Seven patients had a worsening TCD longitudinally. Six of these patients were initially on blood transfusion (BTx); 4 patients stopped transfusion but 2 of those continued with alternative treatments. Two of the 7 patients had a normal baseline TCD (on blood transfusion) and had headaches; one had had stroke and from a normal TCD progressed to undetectable MCA; the other patient progressed to decreased MCA blood flow velocity less than 70 cm/sec (he stopped BTx and continued with Hydroxyurea). Five patients had an abnormal baseline TCD; 3 of these patients (on Btx, one stopped, another had a bone marrow transplant) presented with anterior territory TIAs, the TCDs of two of them progressed from low MCA velocities <70 cm/sec to an undetectable MCA, and another patient from MCA velocities > 200cm/sec (severe turbulence) to low MCA velocities <70 cm/sec. Of the other 2 patients with abnormal baseline TCD, one

had a posterior TIA (stopped BTx), and the other headaches (no BTx) with low MCA velocities < 70 cm/sec to undetectable MCA.

As can be seen from table 5.9, only 2 patients with a normal baseline TCD had impaired studies at follow-up. Two third of the patients with TIAs and abnormal baseline TCD had worsening of their MCA blood flow. Patients with headaches had a variety of TCD changes, however 2/3 of these patients had unchanged or better TCD at follow-up and 1/3 had impaired MCA flow despite blood transfusion. The asymptomatic patient (on BTx) maintained his TCD pattern throughout.

Change Over Time (n= 17 Sickle Cell Patients) TCD						
Recurrent Neurological Symptoms (n=patients)	**Better (BTx)**		**Unchanged (BTx)**		**Worse (BTx)**	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
Coma (PLKE) [+]						
Stroke[+]						
Anterior Territory TIA (n=6)		**1 (1)** 1 §HU+RV *A*		**2 (2)** *D*		**3 (3)** [1 §] 1 §BMT *I*
Posterior Territory TIA (n=1)						**1 (1)** [1 §] *J*
Seizures (n=1)				**1 (1)** *E*		
Headaches (n=9)		**1** *B*	**2** *C*	**2 (2)** [2 §] *F*	**2 (2)** 1 §HU *H*	**1** *K*
Asymptomatic				**1 (1)** *G*		

Table 5.9. Changes over time on transcranial doppler ultrasound (TCD) in relation to recurrent neurological symptoms and therapies in patients with sickle cell disease.

[+] No comparative data (no initial TCD)
BTx: Blood transfusion therapy
PLKE: Posterior leukoencephalopathy
TIA: Transient ischaemic attack
HU Number of sickle cell patients on Hydroxyurea therapy
§ Number of patients who stopped blood transfusion therapy during follow-up
§HU Number of patients who stopped blood transfusion therapy and continued with Hydroxyurea during follow-up
§BMT Number of patients who stopped blood transfusion therapy and had bone marrow transplant during follow-up
§HU+RV Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up
No data because no improvement can be demonstrated from a normal TCD

Patients from Appendix Tables 3 and 4 (Patient number):
A: 14 / ***B:*** 17 / ***C:*** 18, 23 / ***D:*** 6,7 / ***E:*** 4 / ***F:3, 20*** / ***G:***11 / ***H:***15, 9 / ***I:***10,12,13 /***J***: 16 / ***K***: 22

5.5.3.1. Predictors of Recurrent Neurological Symptoms and Worsening TCD

At follow-up, fifty percent of patients with TIAs had a worse TCD despite BTx, 60% of patients with headaches had better or unchanged study (with and without BTx), and one patient with seizures remained with the same TCD flow velocities (on BTx).

5.5.3.2. Changes over Time on TCD and Recurrent Neurological Symptoms in SCD-Statistical Analysis

Abnormal transcranial Doppler ultrasound study at follow-up was not significantly related to the presence or absence of recurrent neurological symptoms, including headaches (p=1, Fisher's exact test), or with patients who have only recurrent TIAs or seizures (p=0.63). However, there were weak trends for an association between recurrent TIAs and headaches and worsening TCD (p=0.14 and p= 0.14 respectively, Fisher's exact test). The other recurrent symptoms could not be analysed because the numbers were small.

There was a trend for an association between severity of TCD category at follow-up (from normal to undetectable MCA) and recurrent TIAs or seizures (p=0.095, Mann-Whitney test, table 5.10).

Recurrent TIA or Seizures	**TCD at Follow-Up**					**Total**
	Normal	MCA ≥170 < 200 cm/sec	MCA ≥ 200 cm/sec	MCA veloc< 70 cm/sec & ratio H:L =<0.5	Undetec-table MCA	
No	3			4	2	9
Yes				4	4	8
Total	3	0	0	8	6	17

Table 5.10. Relation between transcranial Doppler ultrasound categories of severity at follow-up and recurrent TIA or seizures.

There was no significant association between TCD category of severity and changes of TCD over time -better, unchanged, worse- (p=0.57 Spearman's rho correlation). However, there were trends for associations between severity of TCD at onset and follow-up and recurrent neurological symptoms (p=0.07 and p=0.1, Spearman's correlations, tables 5.11 and 5.12).

Recurrent Symptom	**Initial TCD**					**Total**
	Normal	MCA ≥ 170 < 200 cm/sec	MCA V ≥200 cm/sec	MCA V < 70 cm/sec & ratio H:L <0.5	Undetec-table MCA	
Asympto-matic				1		1
Headache	4			4		8
Seizures				1		1
Post TIA				1		1
Ant TIA			1	3	2	6
Total	4	0	1	10	2	17

Table 5.11. Relation between recurrent neurological symptoms and TCD categories of severity at onset in sickle cell patients (p=0.07).

Recurrent Symptom	**TCD at Follow-Up**					**Total**
	Normal	MCA V ≥ 170 < 200 cm/sec	MCA V ≥200 cm/sec	MCA V < 70 cm/sec & ratio H:L =<0.5	Undetec-table MCA	
Asympto-matic				1		1
Headache	3			3	2	8
Seizures				1		1
Post TIA					1	1
Ant TIA				3	3	6
Total	3	0	0	8	6	17

Table 5.12. Relation between recurrent neurological symptoms and TCD categories of severity at follow-up in sickle cell patients (p=0.1).

From tables 5.11 and 5.12, most of the patients with headaches who had had previous normal studies maintained their normal TCD pattern at follow-up, in contrast to patients with more vascular symptoms such as TIAs or seizures whose TCD studies did not improve despite blood transfusion. These patients maintained unchanged studies with decreased MCA flow velocities (very severe arterial stenosis) or undetectable MCA (probable artery occlusion) or progressed from an abnormal TCD baseline pattern to artery occlusion. One patient with MCA velocities > 200 cm/sec (severe arterial stenosis) progressed to decreased flow velocities (increased artery stenosis leading to occlusion).

5.6. Results III: Perfusion Abnormality and Recurrent Neurological Symptoms

5.6.1. Association of Perfusion Abnormality and Recurrent Neurological Symptoms

Twenty-three sickle cell patients underwent perfusion MRI (dynamic susceptibility contrast-MRI) with intravenous bolus of Gadolinium (Gd bolus tracking) at onset and they had repeat studies after a mean of 2 years (range 8 month to 3.5 years). The patients and their initial and follow-up perfusion MRI studies are described in Appendix tables 3 and 4 and 5.13 respectively.

At the end of the study (table 5.13), 6 patients had improved perfusion MRI, 9 had unchanged perfusion MRI and 8 patients had a worsening of their perfusion studies. Perfusion abnormalities consisted mainly of a region of increased mean transit time (MTT) of the passage of the IV Gd bolus or decreased cerebral blood flow (CBF).

The six patients with sickle cell disease who had improved perfusion MRI at follow-up, all had an abnormal baseline perfusion MRI and all were initially on blood transfusion therapy (BTx). Two patients had recurrent anterior territory TIAs: one patient had had stroke and had bilateral regions of perfusion abnormalities in the MCA/ACA territory,

despite having a previous unilateral cerebral infarction; at follow-up he had a regional improvement bilaterally in the same vascular territory. The other patient had had previous TIAs and covert infarctions on MRI, and bilateral perfusion abnormalities in the MCA/PCA territories; he had a regional improvement in the parietal-occipital areas, and had undergone revascularisation. One of the 6 patients had recurrent posterior territory TIAs (bilateral covert infarction; stopped BTx), 1 had seizures (bilateral stroke, on BTx) and 1 headaches (stroke, on BTx); these 3 patients had abnormal regional perfusion in the MCA/PCA territory beyond the area of infarction, and they had an improvement in regional perfusion in the area adjacent to the infarction, which was previously abnormal. One patient had severe headaches with normal MRI, and had extensive perfusion abnormalities in the MCA territory bilaterally; this patient had a dramatic improvement in the abnormal regional perfusion parameters after a year on blood transfusion. However, after stopping treatment he had new bilateral regions of perfusion abnormality in the temporal-occipital areas and he continued to have less severe headaches (patient no. 20, Appendix tables 3 and 4).

Nine patients had unchanged perfusion MRI during follow-up. Seven out of 9 patients had had a normal baseline study, which persisted as normal throughout, and continued to have headaches; only one patient was on BTx and another on Hydroxyurea (HU) therapy. Two of the 9 patients had abnormal baseline MRI perfusion which remained unchanged, and both patients were on BTx initially. One patient had recurrent anterior TIAs (covert infarcts, stopped BTx) and had bilateral perfusion abnormalities in the frontal-parietal areas. The other patient (covert infarct, BTx) had abnormal unilateral perfusion in the parietal subcortical in the infarct area and beyond, and he remained asymptomatic (figure patient with unchanged perfusion).

Eight of 23 patients had a worsening in their perfusion MRI studies at follow-up. One patient had a normal baseline study, and had headaches (he stopped BTx and continued with HU); at follow-up he had an unilateral region of perfusion abnormality in the cortical temporal region.

Seven out of 8 patients with worse perfusion MRI had abnormal baseline studies and they were all initially on blood transfusion. One patient had coma (PLKE) and new cerebral infarct (on BTx, previous stroke) and another patient re-stroked (previous

stroke, she stopped BTx due antibody formation); both patients had progression of perfusion abnormality, which was already beyond the infarct area, to new areas of abnormal regional perfusion in the MCA or MCA/PCA territories. Three patients presented with anterior territory TIAs, of whom 2 patients had history of stroke and presented with new cerebral atrophy on MRI (one BTx, another stopped BTx and had bone marrow transplant) and one had previous TIAs, seizures and covert infarctions on MRI (BTx). All of these patients had extension of perfusion abnormality beyond the infarct area and also new areas of abnormal cerebral perfusion ipsilaterally (n=2) and contralaterally (n=1). Two other patients, who had had stroke, manifested recurrent headaches and were on blood transfusion, had extension in the MCA/ACA (n=1) and PCA/MCA (n=1) territories of the regional perfusion abnormalities beyond the infarct area and now in contralateral regions; the patient with abnormal PCA/MCA perfusion, which led to occipital cortical atrophy, also had decreased unilateral vision. In summary, 4 of 8 patients who had worsening of perfusion also presented with worsening of MRI (2 patients new infarct and 2 new cerebral atrophy).

As can be seen from inspection of Appendix tables 3 and 4, there is a general consistency between the anatomical location of perfusion abnormality (involving cortical/subcortical areas) and recurrent neurological symptoms of demonstrated vasculopathy/ischaemic cause (stroke, TIAs and seizures). However, there was no demostrated association between headaches and perfusion abnormality as the majority of these patients had normal perfusion studies at follow-up, with the exception of a few patients.

From table 5.13, only one patient with a normal baseline had a worse perfusion MRI at follow-up. All the patients whose perfusion status deteriorated were on blood transfusion initially and had an abnormal baseline perfusion MRI. The three patients who improved their cerebral perfusion were on blood transfusion initially, although 2 stopped BTx and another had revascularisation and continued on HU. Fifty percent of patients with anterior TIAs had worse perfusion studies despite blood transfusion. Sixty-three percent (7/12) of patients with headaches had normal perfusion and continued unchanged during follow-up.

Change Over Time (n= 23 Sickle Cell Patients) Perfusion MRI (DSC-MRI)						
Recurrent Neurological Symptoms (n=patients)	**Better (BTx)**		**Unchanged (BTx)**		**Worse (BTx)**	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
Coma (PLKE) (n=1)						**1 (1)** *I*
Stroke (n=1)						**1 (1)** [1§] *J*
Anterior Territory TIA (n=6)		**2 (2)** [1§HU+RV] *A*		**1 (1)** [1§] *F*		**3 (3)** [1§BMT] *K*
Posterior Territory TIA (n=1)		**1 (1)** [1§] *B*				
Seizures (n=1)		**1 (1)** *C*				
Headaches (n=12)		**2 (2)** [1§] *D*	**7 (1)** [1§ 1 HU] *E*		**1 (1)** [1§HU] *H*	**2 (2)** *L*
Asymptomatic (n=1)				**1 (1)** *G*		

Table 5.13. Perfusion MRI over time and recurrent neurological symptoms in sickle cell patients and their treatments.

BTx: Blood transfusion therapy

PLKE: Posterior leukoencephalopathy

TIA: Transient ischaemic attack

[HU] Number of sickle cell patients on Hydroxyurea therapy

[§] Number of patients who stopped blood transfusion therapy during follow-up

[§HU] Number of patients who stopped blood transfusion therapy and continued with Hydroxyurea during follow-up

[§BMT] Number of patients who stopped blood transfusion therapy and had bone marrow transplant during follow-up

[§HU+RV] Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up

■ No data because no improvement can be demonstrated from a normal perfusion MRI study.

Patients from Appendix Tables 3 and 4 (Patient number): ***A:*** 7, 14 / ***B:*** 16 / ***C:*** 4 / ***D***: 2, 20 / ***E:*** 3, 17,18,19, 21, 22, 23 / ***F:*** 12 / ***G:*** 11 / ***H:*** 15 / ***I:*** 1 /***J***: 8 / ***K***: 6,10,13 / ***L***: 5, 9

5.6.2. Recurrent Neurological Symptoms: Predictors of Perfusion Abnormality

Most sickle cell patients with coma (PLKE) and recurrent stroke had a worsening of their cerebral perfusion despite BTx (table 5.14). Most of the patients with headaches maintained normal perfusion throughout without BTx. Patients with recurrent seizures and posterior TIA had unchanged or improved cerebral perfusion on blood transfusion therapy, although a few of them stopped treatment. Patients with anterior TIAs had a range of changes in mean transit time (MTT) over time (table 5.13).

There was an association between patients' severity of central nervous system events at presentation and initial perfusion abnormality as evidenced by the parameter of increased mean transit time (MTT) of the passage of the IV Gd bolus (table 5.14, p=0.007, Spearman's correlation), and there was a trend for an association with decreased cerebral blood flow (CBF; p=0.07, Spearman's correlation). As shown in table 5.14, patients who presented with coma, stroke or TIAs had increased MTT, whereas most of the patients with headaches or seizures had normal initial perfusion. For this analysis, 22 SCD patients were included as one patient presented with increased initial CBF on her perfusion MRI (the only patient of this series) and is described as a case report here.

CNS Events at Onset	**Initial Perfusion MRI (MTT)**				**Total**
	Normal MTT	MTT +	MTT ++	MTT +++	
Headache	5			1	6
Seizures	1				1
Posterior TIA	1		1		2
Anterior TIA	1		1	1	3
Stroke	2		1	4	7
Coma			1	2	3
Total	10	0	4	8	22

Table 5.14. Relation between CNS events at presentation and initial perfusion MRI (mean transit time, MTT) in SCD patients. *Regional MTT* +: mild ; + +: moderate; + + +: severe regional increased MTT.

Only one patient, an eight-year old girl with homozygous sickle cell anaemia (HbSS), had initially increased cerebral blood flow. She presented with anterior territory TIA at onset and, subsequently continued to have recurrent TIAs, seizures and headaches despite treatement with exchange transfusion programme and anticonvulsants. She had had covert infarcts in the right corona radiata on her first MRI which remained unchanged, with cerebrovascular disease (CVD) on her initial MRA (severe right middle cerebral artery (MCA) stenosis, which progressed to MCA occlusion plus collaterals; and severe turbulence in the left anterior cerebral artery, which normalised at follow-up) in association with persisting critical maximum MCA velocities > 200 cm/sec in the right MCA. She showed cerebral perfusion abnormality on her first perfusion MRI, but, interestingly, she had a moderate regional increase in cerebral blood flow and cerebral blood volume in the right frontal/parietal regions with normal mean transit time, and greatly increased CBF, moderatly increased CBV and normal MTT in the right temporal areas. On her final perfusion MRI, the areas with previously increased CBF changed to mildly- to- moderately decreased CBF in the frontal/parietal regions, but MTT became moderately increased in the same regions; in contrast the greatly increased CBF in the right temporal region persisted throughout time but the MTT became abnormal (greatly increased) in this area. This regionally increased CBF and CBV might have been related to areas of hyperaemia secondary to compensatory vasodilatation in association with critical stenosis in this patient, perhaps providing blood flow to these areas and maintaining a normal mean transit time initially. However, it seems that this compensatory mechanism failed over time as CBF was decreased on the patient's final perfusion MRI, although she remained on chronic blood transfusion. Increased CBF and/or CBV might be the first indicators of tissue at risk before regional CBF decreases.

5.6.3. Changes over Time on Perfusion MRI and Neurological Symptoms in SCD-Statistical Analysis

Abnormal perfusion at follow-up was not significantly associated with presence or absence of recurrent neurological symptoms and with recurrent stroke, TIA or seizures (p=1 and p=0.65, Fisher's exact test). Recurrent *headaches* were significantly associated with *unchanged* perfusion MRI at follow-up (p=0.03, Fisher's exact test). Recurrent TIAs were not significantly associated with unchanged or worsening

perfusion MRI (p=0.22, and p=1 respectively, Fisher's exact test). Other recurrent symptoms could not be analysed statistically because of the small numbers.

Recurrent TIAs, seizures or stroke were not significantly associated with worsening perfusion MRI over time (p=0.73, Mann-Whitney test). Severity of recurrent neurological symptoms was not significantly associated with worsening of perfusion MRI over time (p=0.4, Spearman's test).

There was a significant association between the severity of central nervous system events at presentation and initial perfusion abnormality for the parameter of increased mean transit time (p= 0.007, Spearman's correlation, table 5.14), and there was a trend for an association with decreased cerebral blood flow (CBF, p=0.07).

5.7. Results IV: Change over Time among Magnetic Resonance Studies and Transcranial Doppler Ultrasound in Sickle Cell Patients

The relationship between changes over time of the different MR investigations and TCD at onset and follow-up are shown in table 5.15.

All the sickle cell patients with worsening MRI, MRA, TCD or Perfusion MRI had baseline abnormal studies. Most of the patients with worsening studies over time were on chronic blood transfusion therapy. Progression of abnormality over time was greatest for MRA, followed by perfusion MRI, and MRI and TCD in equal numbers.

Most of the patients (87 to 96% depending on the investigation) with normal baseline studies at onset remained normal through time. Between 60 and 66% of the patients with normal MRI and MRA, and 90 % of the patients with normal TCD and perfusion MRI who had had normal baseline studies remained normal without having received blood transfusion therapy.

Patients who had abnormal baseline MRI, MRA, TCD and perfusion MRI and remained with unchanged studies at follow-up were all on blood transfusion therapy at onset; very

few patients stopped treatment.A small proportion of the sickle cell patients showed improvement in their investigations (with exception of MRI) and they were initially on blood transfusion therapy. At follow-up, 26% of patients who had had abnormal baseline perfusion MRI had improved cerebral perfusion, whereas 17% and 8% of patients showed better MRA and TCD respectively in their final studies. More patients had better cerebral perfusion (better perfusion MRI) compared with the improvement of the other investigations.

Change Over Time (n=Sickle Cell Patients)						
Investigations	**Better (BTx)**		**Unchanged (BTx)**		**Worse (BTx)**	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
MRI (n=23)			**9 (3)** [2§ 1HU 1§HU]	**8 (8)** [1§ 1§HU+RV 1§BMT]	**1 (1)** [1§]	**5 (5)** [1§]
MRA (n=23)		**4 (3)** [3§]	**7 (3)** [1§ 1HU 1§HU]	**3 (3)**	**1**	**8 (8)** [1§ 1§BMT 1§HU+RV]
TCD (n=17)		**2 (1)** [1§HU+RV]	**2**	**6 (6)** [2§]	**2 (2)** [1§HU]	**5 (4)** [2§ 1§BMT]
Perfusion MRI (n=23)		**6 (6)** [2§ 1§HU+RV]	**7 (1)** [1§ 1HU]	**2 (2)** [1§]	**1 (1)** [1§HU]	**7 (7)** [1§ 1§BMT]

Table 5.15. Change over time of MRI, MRA, transcranial Doppler Ultrasound and perfusion MRI in relation to recurrent neurological symptoms and therapy.
BTx: Blood transfusion therapy
TIA: Transient ischaemic attack
HU Number of sickle cell patients on Hydroxyurea therapy
§ Number of patients who stopped blood transfusion therapy during follow-up
§HU Number of patients who stopped blood transfusion therapy and continued with Hydroxyurea
§BMT Number of patients who stopped blood transfusion therapy and had bone marrow transplant
§HU+RV Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up

No data because no improvement can be demonstrated from a normal study or from an MRI study showing the presence of lesions

5.7.1. Perfusion Abnormality and MRI: Statistical Analysis

Changes of structural MRI and perfusion MRI over time (better, unchanged or worse) were not significantly associated (p=0.3, Spearman's correlation test). Change of perfusion MRI over time was not significantly associated with increasing infarct size and infarct number at follow-up (p=0.3 and p=0.6, Spearman's correlations).

In relation to perfusion MRI parameters, infarct number and infarct size on the sickle cell patients' initial MRI studies were significantly associated with regionally decreased CBF (p=0.004 and p=0.012 respectively) and increased MTT (p=0.075 and p=0.05 respectively, Spearman's correlations) on the initial perfusion MRI scans. Follow-up MRI and perfusion MRI studies also showed significant associations between infarct size and number with decreased CBF (p<0.0001 and p=0.02 respectively) and between infarct size and increased MTT (p=0.027) on the final scans. There was also a weak trend for association between infarct number and MTT on the final scans (p=0.13, Spearman's correlations).

5.7.2. Perfusion Abnormality and MRA: Statistical Analysis

Changes of MRA and perfusion MRI over time were significantly associated (p=0.03, Spearman's correlation).

In relation to perfusion MRI parameters, there were significant associations between initial grade of MRA turbulence and decreased cerebral blood flow (p <0.0001) and increased MTT (p=0.02). At follow-up, grade of MRA turbulence was also significantly associated with final decreased CBF (p <0.0001) and increased MTT (p=0.003, Spearman's correlations).

Initial MRA turbulence severity was significantly associated with initial infarct size and number (p=<0.0001 and p=0.006 respectively, Spearman's correlations).

However, changes over time of MRI and MRA were not significantly associated (p=0.63, Spearman's correlation).

In addition, severity of MRA turbulence at follow-up was significantly associated with infarct size and infarct number on the final MRI scans ($p<0.0001$ and $p=0.014$, Spearman's correlations). Final MRA studies showed that sickle cell patients who had at least one infarct had moderate or severe vessel turbulence or artery occlusion. However, patients with multiple cerebral infarcts (which include covert [silent] infarcts or the association of covert and overt infarcts) had a wide range of MRA turbulence grades (table 5.16).

Infarct Number at Follow-Up	**MRA Turbulence at Follow-Up**						**Total**
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
No Infarct	7	1	1				9
1 Infarct						1	1
2 Infarcts			1		1	2	4
3 Infarcts				1			1
Multiple Infarcts	1	2	2	1	1	1	8
Total	8	3	4	2	2	4	23

Table 5.16. Relation between MRA turbulence and infarct **number** at follow-up in sickle cell patients.

5.7.3. Perfusion Abnormality and Transcranial Doppler: Statistical Analysis

There was a trend for an association between changes of perfusion MRI and transcranial Doppler (TCD) ultrasound over time ($p=0.05$). There was a significant correlation between changes of perfusion MRI and increasing category of TCD severity in the initial study ($p=0.01$, Spearman's correlation, table 5.17). However, there was no association with increasing category of TCD severity at follow-up ($p=1$, Spearman's correlation, table 5.18).

The relation between initial TCD and follow-up cerebral perfusion demonstrated different natural histories for perfusion abnormality. Sickle cell patients who initially

had mean maximum middle cerebral artery (MCA) velocities > 200 cm/sec or decreased mean MCA velocities < 70 cm/sec (and lowest/highest velocity ratio ≤ 0.5) showed a worsening in their perfusion MRI studies at follow-up. Other patients who also had decreased MCA velocities < 70 cm/sec or undetectable MCA had improvement in cerebral perfusion or unchanged perfusion throughout (table 5.17). For the 4 patients with normal TCD at onset, 2 had worse perfusion and 2 were unchanged.

Initial TCD	**Changes in Cerebral Perfusion over Time**			**Total**
	Better	Unchanged	Worse	
Normal		2	2	4
MCA veloc ≥ 170 < 200 cm/sec				0
MCA vel ≥ 200 cm/sec			1	1
MCA veloc< 70 cm/sec & ratio H:L <0.5	3	5	2	10
Undetectable MCA	2			2
Total	5	7	5	17

Table 5.17. Relation between change of perfusion MRI over time and TCD category at onset.

TCD at Follow-Up	**Changes in Cerebral Perfusion over Time**			**Total**
	Better	Unchanged	Worse	
Normal		3		3
MCA vel. ≥ 170 < 200 cm/sec				0
MCA vel ≥ 200 cm/sec				0
MCA veloc< 70 cm/sec & ratio H:L<0.5	3	2	3	8
Undetectable MCA	2	2	2	6
Total	5	7	5	17

Table 5.18. Relation between change of perfusion MRI over time and TCD category at follow-up.

In relation to perfusion MRI parameters (CBF and MTT), the severity of increased MTT and TCD categories at onset were significantly associated (p= 0.02, Spearman's correlation) and there was a trend for an association between initial decreased CBF and TCD (p=0.12). At follow-up, TCD severity was also significantly associated with increased MTT (p=0.015) and decreased CBF (p=0. 046).

5.7.3.1 Relation between TCD and MRI

Changes over time of MRI and TCD were not significantly associated (p=0.55, Spearman's correlation).

There was no association between severity of the initial TCD and infarct size (p=0.39) but there was a significant association between infarct number on the initial MRI and TCD (p=0.04, Spearman's correlations). TCD category was also significantly associated with infarct size and infarct number at follow-up (p=0.04 and p=0.03 respectively, Spearman correlations, tables 5.19 and 5.20).

Infarct Size At Follow-Up	**TCD at Follow-Up**					**Total**
	Normal	MCA V ≥170 < 200 cm/sec	MCA V ≥ 200 cm/sec	MCA V < 70cm/sec & ratio H:L =<0.5	Undetec-table MCA	
No Infarct	4			3	2	9
< 1cm (Small)				3	3	6
1-5 cm- (Moderate)				3	2	5
> 5 cm (Large)				1	2	3
Total	4	0	0	10	9	23

Table 5.19. Relation between transcranial Doppler ultrasound and infarct **size** at **follow-up** in sickle cell disease.

Infarct Number at Follow-Up	TCD at Follow-Up					Total
	Normal	MCA V ≥ 170 < 200 cm/sec	MCA V ≥200 cm/sec	MCA V < 70 cm/sec & ratio H:L <0.5	Undetec-table MCA	
No Infarct	4			3	2	9
1 Infarct				1		1
2 Infarcts				2	2	4
3 Infarcts					1	1
Multiple Infarcts				4	4	8
Total	4	0	0	10	9	23

Table 5.20. Relation between transcranial Doppler ultrasound and infarct **number** at **follow-up** in sickle cell disease.

Sickle cell patients who had abnormal TCD at follow-up with low MCA velocities or undetectable MCA had cerebral infarcts on their final MRI scans, with a range of infarct number and size at follow-up. Patients were more likely to have multiple and larger infarcts if they had undetectable MCA (p=0.03, Spearman's correlation). However, patients without infarcts had a variety of TCD patterns, with a slight preponderance of normal TCD studies.

5.7.3.2. Relation between TCD and MRA

Change of TCD over time was not significantly associated with changes on MRA (p=0.78, Spearman's correlation).

Categories of TCD severity and MRA turbulence on the initial studies were not significantly associated (p=0.7, Spearman's correlation), but there was a trend for association between TCD and MRA turbulence at follow-up (p=0.1, Spearman's correlation). Sickle cell patients with low maximum MCA velocities less than 70 cm/sec (and abnormal lowest/highest ipsilateral MCA velocity ≤ 0.5) or undetectable MCA had different grades of MRA turbulence or arterial occlusion. Only one third of the patients with normal MRA studies had normal TCD, possibly due to technical difficulty with the ultrasound window (table 5.21).

MRA Turbulence at Follow-Up	TCD at Follow-Up					Total
	Normal	MCA ≥ 170 and < 200 cm/sec	MCA V ≥200 cm/sec	MCA veloc< 70 cm/sec & ratio H:L =<0.5	Undetec-table MCA	
Normal	3			3	2	8
Mild	1			1	1	3
Moderate				2	2	4
Severe					2	2
Occlusion				2		2
Occlusion + Collateral				2	2	4
Total	4	0	0	10	9	23

Table 5.23. Relation between MRA turbulence and TCD at follow-up in SCD.

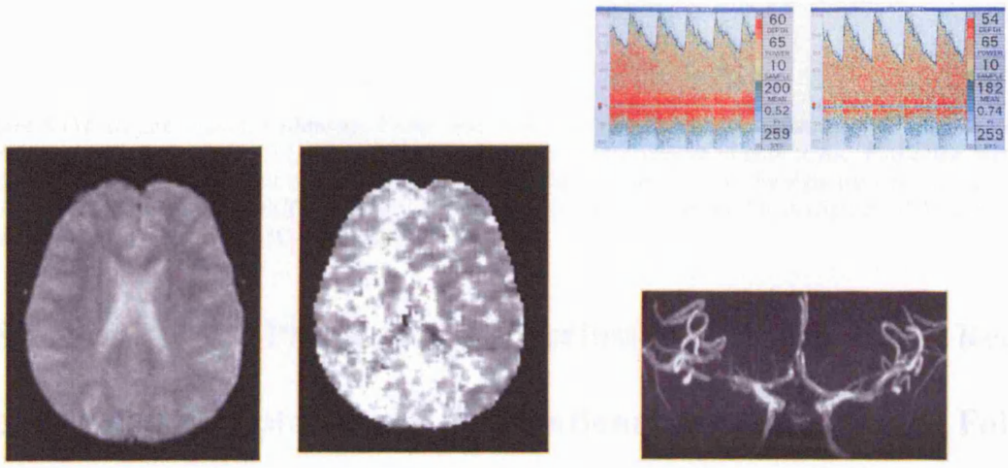


Figure 5.11a. ***Initial***. 11 year-old, male,SCA. Chest crisis followed by pain in right eye, severe headache and blurred vision. Normal ***MRI*** . ***Perfusion MRI:*** severe increased mean transit time of the passage of Gadolinium in the right MCA territory & left temporal occipital region (right >>left). ***MRA*** showed cerebrovascular disease (severe turbulence in middle cerbral arteries [MCA]). ***TCD***: right MCA velocities 200 cm/sec. R: right; L: left.

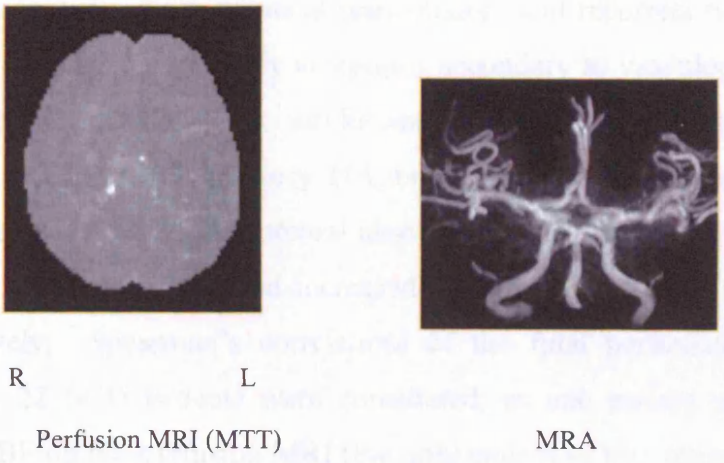


Figure 5.11b. ***One year post-blood transfusion***. Normal ***Perfusion MRI*** (normal MTT), ***MRI*** and ***TCD***. ***MRA***: improved turbulence in MCAs . No symptoms, stopped blood transfusion

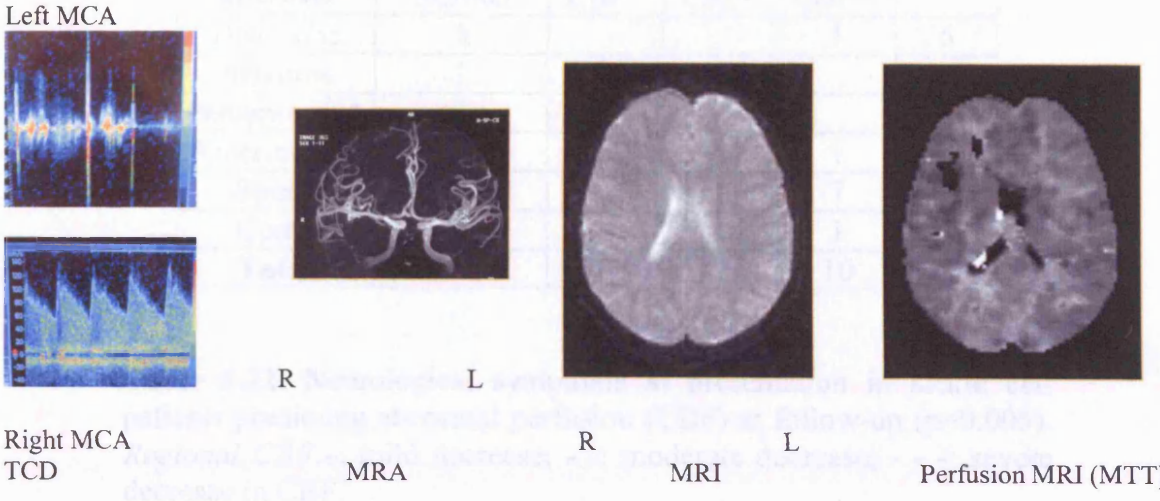


Figure 5.11c. (figure above). ***Follow-up.*** 2 years after stopping blood transfusion. Patient with learning difficulties, occasional headaches. Normal T2-w ***MRI, MRA***: new regions of turbulence in both ICAs, ***Perfusion MRI*** : moderate increased mean transit time of the passage of IV Gadolinium bolus in the right temporal-occipital and left occipital bordezone regions. ***TCD***: decreased velocities < 70 cm/sec in the left MCA (figure: TCD above) and normal velocities in the right MCA (figure: TCD below).

5.8. Results V: Predictors of Perfusion Abnormality, Recurrent Neurological Symptoms, and Conventional Neuroimaging at Follow-up

5.8.1. Predictors of Cerebral Perfusion Abnormality at Follow-up

5.8.1.1. Neurological Symptoms at Presentation and Recurrent Neurological Symptoms

Central nervous system events at presentation and recurrent neurological symptoms in patients with SCD, especially symptoms secondary to vasculopathy/ ischaemia such as initial anterior territory TIA, stroke and coma at presentation, and recurrent stroke, coma (PLKE), anterior territory TIA and seizures during follow-up were significantly associated with decreased cerebral blood flow (CBF, p= 0.005 [table 5.22] and p=0.017 [table 5.23] respectively) and increased mean transit time (MTT, p=0.002 and p=0.03 respectively, Spearman's correlation) of the final perfusion MRI studies. For this analysis, 22 SCD patients were considered, as one patient presented with increased initial CBF on her perfusion MRI (the only patient of this series, whose case report was described in another section above).

CNS Events at Onset	**Perfusion MRI (CBF) at Follow-up**				**Total**
	Normal	CBF -	CBF--	CBF---	
Headache	5			1	6
Seizures	1				1
Posterior TIA	2				2
Anterior TIA	1		1	1	3
Stroke				7	7
Coma	1		1	1	3
Total	10	0	2	10	22

Table 5.22. Neurological symptoms at presentation in sickle cell patients predicting abnormal perfusion (CBF) at follow-up (p=0.005). *Regional CBF* -: mild decrease; - -: moderate decrease; - - -: severe decrease in CBF.

Recurrent Symptom	Perfusion MRI (CBF) at Follow-up				Total
	Normal	CBF -	CBF--	CBF---	
Asymptomatic	1				1
Headache	8			4	12
Seizures				1	1
Posterior TIA	1				1
Anterior TIA			1	4	5
Stroke				1	1
Coma (PLKE)			1		1
Total	10	0	2	10	22

Table 5.23. Recurrent neurological symptoms predicting abnormal perfusion (CBF) at follow-up in SCD patients (p=0.017). *Regional CBF* -: mild decrease; - -: moderate decrease; - - -: severe decrease in CBF.

5.8.1.2. MRI Abnormality at Onset

Size and number of the cerebral infarct on the initial T2-weighted MRI scan were significantly associated with persisting abnormal perfusion MRI parameters at follow-up (for CBF, p<0.0001 and p=0.002 respectively; and for MTT, p=0.01 and p=0.02, respectively). A persisting decreased regional cerebral blood flow at follow-up was associated both with increasing infarct size (table 5.24), especially in patients who had moderate (1-5 cm diameter) or large infarcts (> 5 cm diameter) on their initial T2-weighted MRI scans; and also with increasing infarct number (more than 1 infarct; table 5.25) on the patients' initial MRI studies.

Initial Infarct Size	Final Perfusion MRI (CBF)				Total
	Normal	CBF -	CBF--	CBF---	
No infarct	9			1	10
< 1cm-Small			2	2	4
1-5 cm-Moderate	1			4	5
> 5 cm-Large				3	3
Total	10	0	2	10	22

Table 5.24. MRI predictors of final perfusion abnormality (CBF) in sickle cell patients. **Initial** infarct **size** on T2- weighted MRI (p=<0.0001). *Regional CBF* -: mild decrease; - -: moderate decrease; - - -: severe decrease in CBF.

Initial Infarct Number	Final Perfusion MRI- CBF				Total
	Normal	CBF -	CBF--	CBF---	
No infarct	9			1	10
1 Infarct				1	1
2 Infarcts				3	3
3 Infarcts				1	1
Multiple Infarcts	1		2	4	7
Total	10	0	2	10	22

Table 5.25. MRI predictors of final perfusion abnormality (CBF) in sickle cell patients. **Initial** infarct **number** on T2- weighted MRI (p=0.002). *Regional CBF* -: mild decreased; - -: moderate decreased; - - -: severe decreased CBF.

5.8.1.3. MRA Turbulence at Onset

Arterial blood flow turbulence on the patients' initial MR angiogram was significantly associated with persisting cerebral perfusion abnormality at follow-up (for CBF, p= <0.0001; for MTT, p= 0.01). Patients who had an initial moderate or severe turbulence or arterial occlusion on MRA had severely decreased cerebral blood flow at follow-up. However, patients who had moderate turbulence on MRA also had normal perfusion or moderate decrease in CBF. Two patients with arterial occlusion and collaterals had severely decreased CBF at follow-up, despite having developed a compensatory collateral circulation. By contrast, most of the patients who had normal MRA or mild arterial turbulence at onset had a normal cerebral perfusion over time (table 5.26).

Initial MRA Turbulence	Final Perfusion MRI- CBF				Total
	Normal	CBF -	CBF--	CBF---	
Normal	7		1		8
Mild	1				1
Moderate	2		1	3	6
Severe				3	3
Occlusion				2	2
Occlusion + Collateral				2	2
Total	10	0	2	10	22

Table 5.26. MRA grade of turbulence vs perfusion abnormality at follow-up in SCD. *Regional CBF* -: mild decrease; - -: moderate decrease; - - -: severe decrease in CBF.

5.8.1.4. Perfusion MRI at Onset

Sickle cell patients who had normal or abnormal cerebral blood flow (CBF) initially, generally had the same result at follow-up. Patients with normal, or moderately or severely decreased CBF had a strong tendency to continue with similar parameters throughout time ($p<0.0001$, Spearman's correlation, table 5.27). There was also a significant association between the initial and final perfusion MRIs for mean transit time (MTT), which is inversely proportional to CBF ($p<0.0001$, table 5.28). Initial cerebral blood flow (CBF) and MTT seem to be sensitive predictors of cerebral perfusion longitudinally.

Initial CBF	**Final CBF**				**Total**
	Normal	CBF -	CBF--	CBF---	
Normal	10		1	1	12
CBF –					0
CBF--			1	2	3
CBF---				7	7
Total	10	0	2	10	22

Table 5.27. Predictors of perfusion abnormality in SCD: Cerebral blood flow (CBF) at onset on perfusion MRI. *Regional CBF* -: mild decreased; - - : moderate decreased;
- - -: severe decreased CBF.

Initial MTT	**Final MTT**				**Total**
	Normal	MTT +	MTT ++	MTT +++	
Normal	7		1	2	10
MTT +					0
MTT ++	1		1	2	4
MTT +++				8	8
Total	8	0	2	12	22

Table 5.28. Predictors of perfusion abnormality in SCD: Mean transit time (MTT) at onset on perfusion MRI. *Regional MTT* +: mild ; + +: moderate; + + +: severe regional increased MTT

5.8.1.5. TCD at Onset

There was a weak trend for an association between abnormal TCD at onset and perfusion abnormality at follow-up (for CBF, p= 0.2; and for MTT, p= 0.14, Spearman's correlation). Fifty percent of the sickle patients who had had initially

decreased mean maximum MCA velocities < 70 cm/sec and lowest/highest MCA ratio < 0.5 or undetectable MCA had severely decreased CBF at follow-up. However, 50% of patients with low velocities had normal perfusion at follow-up, perhaps refelecting technical difficulty secondary to the ultrasound window or a reliable cut-off depth for age (with increasing age, the reliable ultrasound depth decreases for obtaining an adequate signal). The majority of the patients with initially normal TCD (3/4) had a normal perfusion MRI over time (table 5.29).

	Final CBF				**Total**
Initial TCD	Normal	CBF -	CBF--	CBF---	
Normal	3			1	4
MCA V ≥ 170 < 200 cm/sec					0
MCA V ≥ 200 cm/sec					0
MCA V < 70 cm/sec & ratio H:L <0.5	5			5	10
Undetectable MCA			1	1	2
Total	8	0	1	7	16

Table 5.29. Initial transcranial Doppler ultrasound vs perfusion abnormality at follow-up in SCD. *Regional CBF* -: mild decreased; - - : moderate decreased; - - -: severe decreased CBF.

5.8.2. Predictors of Recurrent Neurological Symptoms in Sickle Cell Disease

5.8.2.1. Neurological Symptoms at Presentation

There was a significant correlation between the severity of the central nervous system events (CNS) at presentation and the severity of the recurrent neurological symptoms (p=0.04, Spearman's correlation). Patients who had had stroke or transient ischaemic attacks (TIAs) at onset continued to have TIAs during follow-up. Fifty percent of the patients who had had stroke developed recurrent TIAs or seizures, and the only patients who re-stroked or presented in coma (posterior leukoencephalopathy [PLKE]) in this series had previous history of stroke. Alternatively, sickle cell patients who had only headaches at presentation continued with the same type of symptom during follow-up, rather than developing more serious ischaemic symptoms (table 5.30)

CNS Events at Onset	Recurrent Neurological Symptoms						
	Asympto-matic	Headaches	Seizures	Posterior TIA	Anterior TIA	Stroke	PLKE
Headache		6					
Seizures		1					
Posterior TIA		1		1			
Anterior TIA	1				3		
Stroke		2	1		3	1	
Coma		2					1
Total	1	12	1	1	6	1	1

Table 5.30. Central nervous system events at presentation vs recurrent neurological symptoms in sickle cell patients at follow-up.

5.8.2.2. MRI at Onset

There was a significant association between the number of cerebral infarcts on the initial T2- weighted MRI scans and the severity of recurrent neurological symptoms (p=0.002, Spearman's correlation). Increasing number of infarcts (more than 1 infarct) was associated with seizures, anterior territory TIAs, stroke and coma (PLKE). However 3/12 sickle patients who had recurrent headaches presented with 1 or more infarcts on the initial MRI (table 5.31).

There was a trend for association between initial infarct size and severity of recurrent neurological symptoms (p=0.07).

Infarct Number at Onset	Recurrent Neurological Symptoms						
	Asympto-matic	Headaches	Seizures	Posterior TIA	Anterior TIA	Stroke	Coma (PLKE)
No infarct		9		1			
1 Infarct		1					
2 Infarcts		2			2		
3 Infarcts					1		
Multiple Infarcts	1		1		3	1	1
Total	1	12	1	1	6	1	1

Table 5.31. Recurrent neurological symptoms in SCD vs initial infarct **number** on T2- weighted MRI. PLKE: posterior leukoencephalopathy.

5.8.2.3. MRA Turbulence at Onset

There was a trend for association between initial MRA turbulence and recurrent neurological symptoms (p=0.07, Spearman's correlation). Sickle cell patients with recurrent symptoms such as TIAs had moderate to severe turbulence on the initial MRA. The patient who re-stroked had arterial occlusion in her first MRA study, and arterial occlusion was also observed in the patient who had recurrent seizures. In contrast, patients who suffered from recurrent headaches presented with a variety of MRA turbulence grades on the initial studies. However, 7 of 12 patients (58%) with headaches had a normal baseline MRA, and the one patient who remained asymptomatic only showed mild artery turbulence in his first MRA (he had had covert infarctions and was on chronic transfusion, table 5.32).

Initial MRA Turbulence	**Recurrent Neurological Symptoms**						
	Asympto-matic	Headaches	Seizures	Posterior TIA	Anterior TIA	Stroke	Coma (PLKE)
Normal		7					1
Mild	1						
Moderate		3		1	2		
Severe					4		
Occlusion			1			1	
Occlusion + Collateral		2					
Total	1	12	1	1	6	1	1

Table 5.32. MRA turbulence vs recurrent neurological symptoms in SCD. PLKE: posterior leukoencephalopathy.

5.8.2.4. Perfusion MRI Abnormality at Onset

There was trend for an association between initial abnormal cerebral perfusion (increased MTT) and recurrent neurological symptoms (p=0.057, Spearman's correlation) and for abnormal cerebral blood flow (p=0.149). Patients who had recurrent seizures, TIAs, stroke and coma (PLKE) had initial perfusion abnormalities with moderate to severe increased regional MTT. The patient who re-stroked had initially severe increase of the regional MTT (area of infarction and beyond); however, for those patients who had recurrent TIAs, 50% initially presented with moderately and 50% with

severely increased MTT. Eight of 12 patients with recurrent headaches had normal perfusion MRI at onset (table 5.33).

Onset Perfusion MRI (MTT)	**Recurrent Neurological Symptoms**						
	Asympto-matic	Headache	Seizures	Posterior TIA	Anterior TIA	Stroke	Coma (PLKE)
Normal	1	8			1		
MTT +							0
MTT ++				1	2		1
MTT +++		4	1		2	1	
Total	1	12	1	1	5	1	1

Table 5.33. Perfusion MRI parameter (MTT) vs recurrent neurological symptoms in SCD. PLKE: posterior leukoencephalopathy. MTT: +: mild ; + +: moderate; + + +: severe regional increased MTT

5.8.2.5. TCD at Onset

Increasing severity of the initial transcranial Doppler ultrasound patterns showed a trend for an association with severity of recurrent neurological symptoms in patients with SCD (p= 0.07, Spearman's correlation). All the patients who had recurrent seizures and TIAs had abnormal initial TCD patterns (table 5.34). Those patients with velocities > 200 cm/sec or undetectable MCA had further anterior TIAs during follow-up. In contrast, 50% of patients with recurrent headaches had normal TCD and 50% abnormal TCD, perhaps due to technical difficulties (ultrasound window).

Initial TCD	Recurrent Neurological Symptoms					Total
	Asympto-matic	Headaches	Seizures	Posterior TIA	Anterior TIA	
Normal		4				4
MCA V ≥ 170< 200 cm/sec						0
MCA V ≥ 200 cm/sec					1	1
MCA V < 70 cm/sec & ratio L:H =<0.5	1	4	1	1	3	10
Undetecta-ble MCA					2	2
Total	1	8	1	1	6	17

Table 5.34. Transcranial Doppler ultrasound patterns vs recurrent neurological symptoms in SCD.

5.8.3. Predictors of Recurrence of Cerebral Infarction

5.8.3.1. Neurological Symptoms at Onset

Neurological symptoms at presentation were highly associated with infarct size and infarct number at follow-up ($p<0.0001$ and $p=0.007$, Spearman's correlations). Increasing severity of CNS events at presentations were related with increasing size and number of the cerebral infarct on the final MRI studies of the sickle cell patients. It is noteworthy that patients who had headaches as the initial symptom did not have cerebral infarcts during the follow-up (table 5.35 and 5.36).

CNS Events at Onset	Infarct Size at Follow-Up				Total
	No infarct	< 1cm-Small	1-5 cm-Moderate	> 5 cm-Large	
Headache	6				6
Seizures	1				1
Post TIA	1	1			2
Ant TIA		3	1		4
Stroke		1	4	2	7
Coma	1	1		1	3
Total	9	6	5	3	23

Table 5.35. Central nervous system events at presentation vs infarct **size** on T2-weighted MRI at follow-up in SCD.

CNS Events at Onset	Infarct Number at Follow-Up					Total
	No Infarct	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
Headache	6					6
Seizures	1					1
Post TIA	1				1	2
Ant TIA			1		3	4
Stroke		1	2	1	3	7
Coma	1		1		1	3
Total	9	1	4	1	8	23

Table 5.36. Central nervous system events at presentation predicting infarct **number** on T2-weighted MRI at follow-up in SCD.

5.8.3.2. Initial MRI Abnormality

Infarct size and infarct number on the initial T2- weighted MRI scan of the sickle cell patients were significantly correlated with the infarct size and number on the final scans ($p = <0.0001$ and $p=<0.0001$ respectively, Spearman's correlations). From tables 5.37 and 5.38, both parameters seems to be stable longitudinally, although one patient with a normal initial scan had small multiple infarcts at follow-up. However, no changes were seen (tables 5.37 and 5.38) for those sickle cell patients who had recurrent cerebral

infarction because they already had multiple cerebral infarctions at onset, therefore they were kept in the same category for this statistical analysis initially and at follow-up.

Initial Infarct Size	**Infarct Size at Follow-Up**				**Total**
	No infarct	< 1cm-Small	1-5 cm-Moderate	> 5 cm-Large	
No infarct	9	1			10
< 1cm-Small		5			5
1-5 cm-Moderate			5		5
> 5 cm-Large				3	3
Total	9	6	5	3	23

Table 5.39. Relation of **initial** infarct **size** and **follow-up** infarct **size** on T2- weighted MRI in patients with SCD.

Initial Infarct Number	**Infarct Number at Follow-Up**					**Total**
	No Infarct	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
No infarct	9				1	10
1 Infarct		1				1
2 Infarcts			4			4
3 Infarcts				1		1
Multiple Infarcts					7	7
Total	9	1	4	1	8	23

Table 5.40. Relation of **initial** infarct **number** and **follow-up** infarct **number** on T2- weighted MRI in patients with SCD.

5.8.3.3. MRA Turbulence

Severity of MRA turbulence or arterial occlusion at onset was significantly associated with increasing infarct size and infarct number at follow-up ($p<0.0001$ and $p=0.007$, Spearman's correlations). The majority of the patients who had initial arterial turbulence or occlusion on MRA had infarcts of different size and number. However, all the patients with moderate (1-5 cm diameter) or large infarcts (>5 cm diameter), and most

of the patients (13/14) who had 1 or more infarcts, had some grade of turbulence or arterial occlusion on MRA. On the other hand, most of the patients (7/8) who had normal MRA initially did not develop infarcts during follow-up. Patients with moderate and large infarcts, and with 2 or more infarcts had usually MRA grades ranging from moderate turbulence to artery occlusion. However, 2 patients with initial occlusion and collaterals had moderate, not large, infarcts and one or 2 infarcts (instead of 3 or multiple infarcts), which suggests that a collateral circulation might prevent further ischaemia and cerebral tissue damage (tables 5.39 and 5.40)

Initial MRA Turbulence	**Infarct Size at Follow-Up**				**Total**
	No Infarct	< 1cm-Small	1-5 cm-Moderate	> 5 cm-Large	
Normal	7	1			8
Mild			1		1
Moderate	2	2		2	6
Severe		3	1		4
Occlusion			1	1	2
Occlusion + collateral			2		2
Total	9	6	5	3	23

Table 5.41. MRA turbulence at presentation vs infarct **size** on T2-weighted MRI at **follow-up** in SCD.

Initial MRA Turbulence	**Infarct Number at Follow-Up**					**Total**
	No Infarct	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
Normal	7				1	8
Mild					1	1
Moderate	2		2		2	6
Severe			1	1	2	4
Occlusion					2	2
Occlusion + Collateral		1	1			2
Total	9	1	4	1	8	23

Table 5.42. MRA turbulence at presentation vs infarct **number** on T2-weighted MRI at **follow-up** in SCD.

5.8.3.4. Perfusion MRI

Initial cerebral blood flow (CBF) reduction on perfusion MRI was significantly associated with increased infarct size and infarct number on the final MRI scan of the patients (p=0.007 and p=0.049 respectively, Spearman's correlations, tables 5.41 and 5.42). There was also a significant association between initial abnormal (increased) mean transit time (MTT) and final infarct number (p=0.04, Spearman's correlation) with a trend for an association between initial increased MTT and final infarct size (p=0.06).

There was a trend for those patients who had had moderately and severely decreased CBF initially to have 2 or more infarcts of a moderate or large size on their final MRI scans. Eight of 12 patients (67%) with normal perfusion MRI studies initially maintained normal perfusion throughout. Four of 12 patient maintained a normal cerebral perfusion but had multiple infarcts, perhaps because these lesions were too small (sub-cortical, deep watershed white matter lesions) to affect the perfusion MRI parameters, or reflecting the incomplete coverage of the brain in the perfusion MRI data, since only 6 slices could be acquired, and it is therefore possible that the slice positions from which the perfusion data were obtained did not include the small regions of infarction.

Initial CBF	**Infarct Size at Follow-Up**				**Total**
	No infarct	< 1cm-Small	1-5 cm-Moderate	> 5 cm-Large	
Normal	8	2	2		12
CBF –					0
CBF--		1	1	1	3
CBF---	1	2	2	2	7
Total	9	5	5	3	22

Table 5.41. Infarct **size** at **follow-up** on T2-weighted MRI in SCD vs cerebral blood flow (CBF) at onset on perfusion MRI. *Regional CBF* - : mild decrease; - - : moderate decrease; - - - : severe decrease in CBF.

Initial CBF	Infarct Number at Follow-Up					Total
	No Infarct	1 Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
Normal	8			1	3	12
CBF –						0
CBF--			2		1	3
CBF---	1	1	1		4	7
Total	9	1	3	1	8	22

Table 5.42. Infarct **number** at **follow-up** on T2-weighted MRI in SCD vs cerebral blood flow (CBF) at onset on perfusion MRI.
Regional CBF -: mild decrease;
- - : moderate decrease; - - - : severe decrease in CBF.

5.8.3.5. TCD at onset

There was a significant association between initial severity of TCD category and increase in infarct number on the final MRI scans of the sickle cell patients (p= 0.02, Spearman's correlation). Although patients with < 2 infarcts had several TCD patterns, patients with 3 or more infarcts had decreased middle cerebral artery (MCA) velocities < 70 cm/sec and lowest/highest ipsilateral MCA ratio $\leq$ 0.5 or undetectable MCA (table 5.43).

There was no relationship between the initial TCD and follow-up infarct size on MRI (p=0.35).

Initial TCD	Infarct Number at Follow-Up				Total
	No Infarct	2 Infarcts	3 Infarcts	Multiple Infarcts	
Normal	3	1			4
MCA V ≥ 170<200 cm/sec					0
MCA vel ≥ 200 cm/sec		1			1
MCA veloc< 70 cm/sec & ratio L:H <0.5	4	1	1	4	10
Undetectable MCA				2	2
Total	7	3	1	6	17

Table 5.43. Transcranial Doppler ultrasound patterns vs infarct **number** on T2-weighted MRI at **follow-up** in SCD.

5.8.4. Predictors of MRA Turbulence

5.8.4.1. Neurological Symptoms at Presentation and Recurrent Neurological Symptoms

Severity of the central nervous system events at presentation significantly predicted severity of the MRA turbulence at follow-up (p=0.027, Spearman's correlation). In particular, patients who had TIAs, stroke and coma at onset had persistent moderate to severe artery turbulence or occlusion at follow-up (table 5.44). Headaches and posterior territory TIA seem to be the most benign predictors of MRA turbulence, 4/6 patients maintained a normal MRA study, and 2 developed mild to moderate flow turbulence at follow-up. The patients with posterior territory TIA had normal or mild turbulence on MRA over time.

CNS Events at Onset	MRA Turbulence at Follow-Up						Total
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
Headache	4	1	1				6
Seizures	1						1
Post TIA	1	1					2
Ant TIA		1	2			1	4
Stroke				2	2	3	7
Coma	2		1				3
Total	8	3	4	2	2	4	23

Table 5.44. Central nervous system events at presentation vs MRA turbulence at follow-up in SCD.

There was a trend for an association between severity of recurrent neurological symptoms and MRA turbulence at follow-up (p=0.05, Spearman's correlation). Similarly to the CNS at presentation, recurrent neurological symptoms such as anterior territory TIAs and stroke, and also seizures, had the most severe grades of turbulence or occlusion at follow-up, whereas 7/12 of the patients who had recurrent headaches or posterior territory TIAs had normal or mild turbulence. However, 5/12 of patients who had recurrent headaches also had moderate grades of MRA turbulence or occlusion; some of these patients had had stroke and cerebrovascular disease (CVD) at presentation, but it seems that headache as a recurrent symptom has a different predictive value (symptom of a stabilised condition under treatment [i.e. blood transfusion]) for those patients who had severe neurological symptoms at presentation (table 5.45).

Recurrent Symptom	MRA Turbulence at Follow-Up						Total
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
Asymp-tomatic		1					1
Headache	7	1	2			2	12
Seizures					1		1
Post TIA		1					1
Ant TIA			2	2	1	1	6
Stroke						1	1
Coma (PLKE)	1						1
Total	8	3	4	2	2	4	23

Table 5.45. Recurrent neurological symptoms vs MRA turbulence at follow-up in SCD. PLKE: posterior leukoencephalopathy.

5.8.4.2. MRI Abnormality at Onset

There was a significant association between increasing infarct size and infarct number on the patients' initial MRI scans and grade of turbulence on the final MRA scan ($p < 0.0001$ and $p=0.02$ respectively, Spearman's correlations) as is shown in tables 5.46 and 5.47. Those patients who initially had more than 1 infarct and with moderate to large infarcts, tended to have moderate to severe turbulence or artery occlusion at follow-up.

Initial Infarct Size	MRA Turbulence at Follow-Up						Total
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
No infarct	7	2	1				10
< 1cm-Small	1		2	1		1	5
1-5 cm-Moderate		1		1	1	2	5
> 5 cm-Large			1		1	1	3
Total	8	3	4	2	2	4	23

Table 5.46. Initial infarct **size** on T2-weighted MRI vs MRA turbulence at follow-up in SCD.

Initial Infarct Number	MRA Turbulence at Follow-Up						Total
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
No Infarct	7	2	1				10
1 Infarct						1	1
2 Infarcts			1		1	2	4
3 Infarcts				1			1
Multiple Infarcts	1	1	2	1	1	1	7
Total	8	3	4	2	2	4	23

Table 5.47. Initial infarct **number** on T2-weighted MRI vs MRA turbulence at follow-up in SCD.

5.8.4.3. MRA Turbulence at Onset

Severity of MRA turbulence at onset was highly correlated with MRA turbulence at follow-up (p=<0.0001, Spearman's correlation). Most of those patients who had initially normal MRA (7/8) maintained the normal pattern throughout; however those with moderate to severe turbulence progressed to severe turbulence or artery occlusion during the follow-up (table 5.48).

Initial MRA Turbulence	MRA Turbulence at Follow-Up						Total
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
Normal	7	1					8
Mild		1					1
Moderate	1	1	3		1		6
Severe			1	2		1	4
Occlusion					1	1	2
Occlusion + Collateral						2	2
Total	8	3	4	2	2	4	23

Table 5.48. Initial MRA turbulence vs MRA turbulence at follow-up in SCD.

5.8.4.4. Perfusion MRI at Onset

Severity of the perfusion MRI abnormality at onset (reduction of regional CBF or increased MTT) was significantly associated with increasing of turbulence on MRA at follow-up ($p<0.0001$ for CBF and $p=0.019$ for MTT, Spearman's correlations). Those sickle cell patients who initially had moderately to severely decreased regional CBF had moderately to severely turbulence or occlusion on MRA at follow-up, whereas the patients with initial normal perfusion (8/12) had normal cerebral vasculature throughout with 3 patients developing mild turbulence and only one patient with severe turbulence on the final MRA study (table 5.49).

Initial CBF	**MRA Turbulence at Follow-Up**						**Total**
	Normal	Mild	Moderate	Severe	Occlusion	Occlusion + Collateral	
Normal	8	3		1			12
CBF-							0
CBF--			1		1	1	3
CBF---			3	1	1	2	7
Total	8	3	4	2	2	3	22

Table 5.49. MRA turbulence at follow-up vs cerebral blood flow (CBF) at onset on perfusion MRI. *Regional CBF* -: mild decrease; - - : moderate decrease; - - - : severe decrease in CBF.

5.8.4.5. TCD at Onset

TCD abnormality at onset was not a predictor of MRA turbulence at follow-up ($p=0.68$, Spearman's correlation).

5.8.5. Multivariate Model of Statistical Analysis

Worsening of MRA was significantly associated with worsening of perfusion MRI over time ($p= 0.017$) in a univariate analysis by multiple regression in relation to an outcome (worse/not worse perfusion MRI at follow-up). There were trends for associations with the same outcome for worsening TCD ($p=0.056$) and worsening of MRI ($p=0.07$). There

were no significant associations for the presence or not of recurrent neurological symptoms and for the presence of symptoms such as a stroke, seizures and TIAs against worsening or not of cerebral perfusion at follow-up ($p=0.85$ and $p=0.44$ respectively).

A correlation matrix was calculated to assess the level of correlation among the variables because this impacts on the multiple testing issue. Specifically, a common but naive approach to dealing with multiple testing is to control the overall (familywise) error rate by performing a Bonferroni adjustment. This is not always appropriate, however. Firstly, the correction can be conservative when applied to non-independent tests. Secondly, Bonferroni adjustment is not necessarily appropriate if the variables in question are correlated in the sense that each represents an imperfect measure of some underlying (latent) process or outcome. In this case a single Type I error among a set of significant tests is unimportant in those contexts in which all conclusions are based on the overall effect. In this context familywise error rate control is not called for (this issue is discussed at length by Benjamini and Hochberg (1995). Familywise error rate adjustment has not been adopted in this study, because changes in cerebral perfusion are expected to cause a parallel response in several of the MR observables. Correlation among the variables is expected; the resulting multiple tests are not, therefore, conceived as independent.

There was a high correlation (coefficient of ≥ 0.5) among the following variables: cerebral blood flow, infarct size, infarct number and severity of MRA turbulence (table 5.50). Furthermore, perfusion abnormality at follow-up was significantly associated with each of these variables (tables 5.24 to 5.32). This suggests that there is a principal driving disease process leading to the outcome (in this case abnormal perfusion at follow-up). In this context principle component analysis shows that the first principal component accounts for 70% of the total variance associated with the set of variables (dependent predictors), consistent with a major driving process. The second principal component accounts for an additional 15% of the total variance (table 5.51).

Correlation Matrix

Initial MR Investigation	Initial CBF	Initial Infarct size	Initial Infarct number	Initial MRA turbulence
Initial CBF	1.000	.589	.493	.748
Initial Infarct size	.589	1.000	.659	.644
Initial Infarct number	.493	.659	1.000	.478
Initial MRA turbulence	.748	.644	.478	1.000

Table 5.50. Correlation matrix of predictors of cerebral perfusion abnormality at follow-up.

Total Variance Explained

	Initial Eigen-values		
Component	**Total**	**% of Variance**	**Cumulative %**
1	2.810	70.246	70.246
2	.629	15.734	85.979
3	.328	8.199	94.178
4	.233	5.822	100.000

Table 5.51. Principle component analysis of the correlation matrix derived from the initial MR investigations among cerebral perfusion abnormality at follow-up, showing that there is a principal driving disease process that explains 70% of the total variance associated with the set of variables (dependent predictors), consistent with a major driving process (component 1). The second principal component accounts for an additional 15% of the total variance (component 2).

5.9. Summary of Results

5.9.1. Neurological Symptoms, Blood Pressure, Haematological Parameters and Oxygen Saturations

- Neurological symptoms at presentation consisted of: *stroke* in 29% (7/23) of the sickle cell patients, *transient ischaemic attacks* (TIA, including anterior and posterior territory TIAs) in 26% (n=6), *headaches* in 26% (n=6) and *seizures* (n=1).

- Main recurrent neurological symptoms consisted of: *headaches* in 52% (12/23), *TIAs* in 30% (n=7 [26% had anterior territory TIAs]), *recurrent stroke* (n=1), *coma (posterior leukoencephalopathy)* (n=1), and *seizures* (n=1). One patient remained *asymptomatic.* Associated recurrent symptoms were cognitive problems (behaviour/learning difficulties) in 14/23 patients, TIAs, headaches and seizures.
- There were no significant associations between blood pressure measurements (systolic, diastolic and mean arterial pressure) at follow-up and worsening of MRI, MRA, TCD and perfusion MRI. There was only a trend for association between worsening of TCD and low diastolic blood pressure.
- Haematological parameters showed a trend for association between worsening of perfusion and increased white cell count at follow-up. There was a significant association between worsening of MRA and decreased platelet count at onset. There were also trends for association between increased white cell and lymphocyte counts at follow-up and worsening of MRI.
- Day (awake) oxygen saturations measured using pulse oximetry were not significantly associated with the presence of recurrent neurological symptoms and with worsening of MRI, MRA, TCD and perfusion MRI.

5.9.2. Recurrent Neurological Symptoms and Change over Time of MRI, MRA, TCD and Perfusion MRI

5.9.2.1. Recurrent Neurological Symptoms and MRI Change over Time

- 17/23 patients had unchanged MRI: 9/17 had normal baseline and headache was the main recurrent symptom; 8/17 had abnormal baseline, recurrent symptoms were TIAs (n=5) and headaches (n=2) and one patient remained asymptomatic.
- 6/23 patients had worsening of MRI; 5/6 had abnormal baseline MRI. Recurrent symptoms were coma (PLKE, n=1), stroke (1), anterior (1) and posterior (1) territory TIA, seizures (1) and headaches with decreased visual acuity (1).

- There was a trend for an association for patients who had recurrent stroke, TIAs or seizures to have worsening of MRI. Recurrent headaches and anterior territory TIAs were significantly associated with unchanged MRI.
- The severity of recurrent symptoms was significantly associated with MRI changes over time and increasing infarct number, and there was a trend for association with increasing infarct size.

5.9.2.2. Recurrent Neurological Symptoms and MRA Change over Time

- 4/23 sickle cell patients showed improved the anterior cerebral circulation on MRA at follow-up. Recurrent symptoms were anterior (n=1) and posterior (n=1) territory TIAs, and headaches (n=2).
- 10/23 patients had unchanged MRA. Seven out 10 patients had a normal baseline MRA. Neurological symptoms were predominantly headaches (n=6) and coma (PLKE, n=1). 3/10 had abnormal baseline MRA, symptoms consisted of recurrent seizures (n=1) and headaches (n=1); one patient remained asymptomatic.
- 9/23 had worsening MRA, only one patient had a normal baseline MRA (headaches). Recurrent symptoms of the remainding 8 patients were stroke (n=1), TIAs (n=5) and headaches (n=2).
- There was a trend for an association between recurrent stroke, TIAs or seizures and worsening of MRA, and, conversely to be associated for headaches with unchanged MRA.
- The severity of grade of MRA turbulence at follow-up was significantly associated with recurrent stroke, TIAs or seizures, and with changes of MRA over time. There was a trend for association between severity of recurrent neurological symptoms and increasing grade of MRA turbulence.

5.9.2.3. Recurrent Neurological Symptoms and Transcranial Doppler Ultrasound Change over Time

- Two patients had critical mean maximum MCA velocities (> 200 cm/sec) at onset (one of them had also contralateral decreased MCA velocities < 70 cm/sec, and because the latter is a more severe indicator, this patient was

considered for analysis on the basis of a velocity < 70 cm/sec) and these 2 patients did not have a new cerebral infarction during follow-up but they were on chronic blood transfusion. Ten patients (including the patient with contralateral MCA velocities > 200 cm/sec) had decreased mean maximum MCA velocities < 70 cm/sec at onset, 1/10 patients had new infarcts (covert infarcts) and posterior territory TIAs and 2/10 patients had progression of cortical atrophy (focal) at the end of the study.

- 2/17 patients had an improvement of their transcranial Doppler ultrasound (TCD) at follow-up, one patient had recurrent anterior territory TIA and the other had headaches.
- 8/17 patients had unchanged TCD. 2/8 had a normal baseline TCD (recurrent headaches). 6/8 had an abnormal baseline TCD, their recurrent symptoms were anterior territory TIAs (n=2), seizures (1), headaches (2).
- 7/17 patients had a worsening of their TCD. Two patients had a normal baseline TCD and had headaches. Five patients had an abnormal baseline TCD and they presented with anterior (3) and posterior (1) territory TIAs and headaches (1).
- There were trend for associations between worsening TCD and recurrent TIAs, and between worsening TCD and headaches. There was also a trend for association between severity of TCD category and recurrent TIAs or seizures, and between TCD categories and, the severity of recurrent neurological symptoms.

5.9.2.3. Recurrent Neurological Symptoms and Perfusion MRI over Time

- 6/23 sickle cell patients (26%) improved their perfusion MRI at follow-up. Recurrent neurological symptoms were anterior (n=2) and posterior (1) territory TIAs, seizures (1) and headaches (2).
- 9/23 patients (39%) had unchanged perfusion MRI. 7/9 had a normal perfusion MRI baseline and all patients had recurrent headaches. 2/9 had an abnormal baseline study, one patient had anterior territory TIAs and the other remained asymptomatic.

- 8/23 patients (35%) had a worsening of MRI perfusion at follow-up. One patient had a normal baseline perfusion MRI and he had headaches. 7/8 patients had abnormal baseline studies, and their recurrent symptoms were coma (PLKE, n=1), recurrent stroke (1), anterior territory TIAs (3) and headaches (2).
- There was a good neuroanatomical correlation between regional cerebral perfusion abnormality and recurrent neurological symptoms such as stroke, TIAs and seizures.
- Severity of the initial central nervous system events at presentation were significantly associated with an abnormal perfusion parameter (increased MTT) and there was a trend for decreased CBF.
- There was a significant association between recurrent headaches and unchanged perfusion MRI at follow-up; however there were no associations between worsening of perfusion MRI and recurrent stroke, TIAs or seizures, and severity of the recurrent neurological symptoms.

5.9.3. Changes over Time of Perfusion Abnormality in Relation to Magnetic Resonance Studies and Transcranial Doppler Ultrasound

5.9.3.1. Perfusion MRI and T-2 Weighted MRI

- Changes of MRI and perfusion MRI over time were not significantly associated.
- However, there were significant associations between infarct size and infarct number on the initial and final MRI scans, and between decreased CBF and increased MTT at onset and follow-up respectively.

5.9.3.2. Perfusion MRI and MRA- MRA and MRI

- There was a significant association between changes of MRA and perfusion MRI over time.
- Initial grades of turbulence on MRA were significantly correlated with decreased CBF and increased MTT at onset. Final grades of turbulence on

MRA were also significantly correlated with severity of the decrease in CBF and the increase in MTT at follow-up.

- Changes over time of MRI and MRA were not significantly associated, however, severity of MRA turbulence at onset and follow-up were significantly associated with increasing infarct size and number on the initial and final MRI scans respectively.

5.9.3.3. Perfusion MRI and TCD- TCD and MRI- TCD and MRA

- There was a trend for association (nearly significant) between changes of TCD and perfusion MRI over time.
- There was a significant correlation between changes on perfusion MRI and increasing category of TCD severity on the initial study.
- Initial TCD severity and increased MTT were significantly associated (trend for initial decreased CBF). Follow-up TCD was significantly associated with final CBF and MTT.
- Changes over time of MRI and TCD were not significantly associated.
- However, although there was not a significant association between severity of the initial TCD and infarct size, it was significant for infarct number on the initial MRI.
- In addition, TCD severity at follow-up was significantly associated with increased infarct number and infarct size on the final MRI scans.
- Change of TCD over time was not significantly associated with changes on MRA.
- There was a trend for association between severity of TCD categories at follow-up and MRA turbulence grades on the final studies, but there was not association between initial TCD categories and MRA turbulence.

5.9.4. Predictors of Cerebral Perfusion Abnormality, Recurrent Neurological Symptoms and Conventional Neuroimaging at Follow-up

5.9.4.1. Predictors of Cerebral Perfusion Abnormality at Follow-up

- Severity of central nervous system (CNS) events at presentation (such as coma, stroke and anterior territory TIA) and recurrent neurological symptoms (such as recurrent stroke, coma [PLKE], anterior territory TIA and seizures) were significantly associated with abnormal cerebral perfusion parameters at follow-up characterised by decreased regional cerebral blood flow (CBF) and increased mean transit time (MTT).
- Increasing infarct size and number on the initial T2- weighted MRI were significantly associated with decreased regional CBF and increased MTT at follow-up.
- Increasing grade of MRA turbulence at onset was significantly associated with decreasing regional CBF and increasing MTT on the final perfusion MRI.
- Initial decreased regional CBF and increased MTT were significantly associated with the same perfusion MRI parameters at follow-up. In general, sickle cell patients with normal, moderate or decreased CBF (or increased MTT) continued with similar perfusion parameters at follow-up.
- There was a trend for an association between initial TCD categories and abnormal grades of CBF and MTT at follow-up.
- The perfusion MRI parameter cerebral blood flow (CBF) seems to be a more sensitive parameter for prediction of abnormality over time than mean transit time (MTT).

5.9.4.2. Predictors of Recurrent Neurological Symptoms at Follow-up

- There was significant correlations between the severity of the patients' recurrent neurological symptoms and the severity of the initial CNS events, the number of infarcts on the initial MRI and increasing MRA turbulence grade at onset.

- There were trends for associations between initial regional abnormal cerebral perfusion (decreased CBF and increased MTT), initial TCD categories and severity of recurrent neurological symptoms.

5.9.4.3. Predictors of Recurrence of Cerebral Infarction

- Severity of neurological symptoms of the patients at presentation, initial infarct size and infarct number, severity of MRA turbulence and initial decreased regional CBF on perfusion MRI were significantly associated with increasing infarct size and number on the final T2-weighted MRI scans.
- There were also significant associations for increasing regional MTT on the initial perfusion MRI and initial TCD category with the final infarct number, but there were trends for associations for initial MTT and TCD categories with final infarct size.

5.9.4.4. Predictors of MRA Turbulence

- Increasing severity of the CNS events at presentation was significantly associated with increasing grade on MRA turbulence at follow-up. Severity of recurrent neurological symptoms showed a trend for association with final MRA turbulence.
- Initial increased infarct size and number, grade of MRA turbulence, and abnormal perfusion MRI (decreased CBF or increased MTT) were significantly associated with severity of grade of turbulence at follow-up.
- TCD Doppler abnormality at onset was not a predictor of final grade of MRA turbulence.

5.10. Discussion

This study showed that there was progression over time of cerebral perfusion abnormality in more than one third of the patients with sickle cell disease. This progression of abnormal perfusion was associated with the severity of the patients'

recurrent neurological symptoms and progression of cerebrovascular disease (MRA turbulence) and it continued in many cases despite chronic blood transfusion therapy or other alternative therapies.

In addition, the study demonstrated that more than a third of patients had unchanged cerebral perfusion during follow-up, which was associated with less severe recurrent neurological symptoms and mainly unchanged MRI and MRA, with or without blood transfusion therapy (the latter most frequent).

On the other hand, only one quarter of the sickle cell patients had an improvement in cerebral perfusion over time, associated with improvement on MRA turbulence or unchanged MRA at follow-up. These patients were on blood transfusion; they manifested a variety of recurrent neurological symptoms of different grade of severity, but none of them had recurrent stroke.

The progression of cerebral perfusion abnormality and cerebrovascular disease might be associated with infection in addition to the damaging effect of the sickle cell in the vascular endothelium, as increased white cell and thrombocytopenia were associated with changes of cerebral perfusion and MRA over time.

This series was composed of patients with sickle cell disease who had neurological symptoms. As it was highly selected, there was a high prevalence of stroke at onset (29%) in this patients' group in relation to their age comparing with previous epidemiologic studies, where the prevalence was between 8% -11% in patients younger than 19 years (Ohene-Frempong et al 1991 and 1998). In this group there was also a high prevalence of transient ischaemic attacks (26%), especially anterior territory TIAs. A quarter of the patients had headaches, and one patient had seizures, but none were entirely asymptomatic.

Twenty out of 23 patients presented with recurrent neurological symptoms of different grade of severity, despite the fact that 18 out of 23 patients (78%) were initially on blood transfusion. Probably because a high proportion of these patients were on chronic blood transfusion (some of whom stopped while others continued with this therapy), only 3 patients (13%) had new cerebral infarctions on their final MRI studies, compared

with the high proportion of recurrent stroke (up to 67%) in SCD without blood transfusion (Powars et al 1978). Two of the 3 patients had recurrent infarction (8%); one of the sickle cell patients manifested the cerebral infarct as clinical stroke, and another patient as posterior leukoencephalopathy (PLKE) with coma and hypertension. However, the first patient had stopped blood transfusion therapy a year before her final magnetic resonances studies because of the presence of antibody formation against red cells, whereas the second patient was on chronic blood transfusion. The third patient, who had recurrent posterior territory TIAs, stopped blood transfusion during the follow-up, and she had bilateral new infarcts on the final MRI, having had a normal scan previously.

Although new treatments for SCD have been advocated in recent years (Walters et al 1996 and 2000, Ware et al 1999, Wang et al 2001, Harlan et al 2000, Vernet et al 1996, Ganesan et al 2001), the highest proportion of the patients of this study received the classical treatment of chronic blood transfusion. In spite of that, Pegelow showed that the protective effect of blood transfusion therapy was not complete, with a risk of recurrent stroke in sickle cell children receiving chronic blood transfusion up to 4.2/100 patient-years (Pegelow et al 1995). Stroke recurrence despite blood transfusion was also seen in the present study (prevalence of 8%). Only 4 patients received alternative treatments such as Hydroxyurea, revascularisation procedure plus Hydroxyurea, and bone marrow transplant as only therapy (n=1) or an alternative one after stopping blood transfusion (n=3). In addition 4 patients stopped blood transfusion during follow-up for different reasons (poor compliance, autoantibody formation, etc) without receiving any further alternative treatment.

As stroke is the most alarming and long-term disabling complication, and occurs despite receiving an adequate treatment for their inherited condition (Steimberg 1999, Hoppe et al 1998, Ballas et al 2002), this study was focused on trying to explain the cause or causes of recurrent neurological symptoms in SCD through changes of magnetic resonance (MR) technique (specifically cerebral perfusion) and clinical parameters over time, and to relate these changes with the recurrent symptoms. The effect of the different treatments, especially blood transfusion, on cerebral perfusion and other MR modalities will be discussed in chapter 6.

In relation to changes over time among recurrent neurological symptoms, the different magnetic resonance studies, and transcranial Doppler, the study showed that there is a worsening of cerebral perfusion over time in more than a third of patients with sickle cell disease. This worsening of perfusion was associated with the severity of the recurrent neurological symptoms such as coma (PLKE), stroke and anterior territory TIAs. However the less severe symptom of headache was also associated with worsening of cerebral perfusion in a few patients. All these patients were on blood transfusion therapy. In addition, there was a strong association between the localisation of the regional perfusion abnormality and the clinical symptoms (see table 5.14). The initial severity of the symptoms showed a significant association with the degree of abnormal perfusion, in this case represented by the perfusion MRI parameter increased mean transit time (MTT). Recurrent neurological symptoms were also significantly associated with the abnormal perfusion parameters of decreased regional cerebral blood flow (CBF) and increased MTT at follow-up. Worsening of perfusion MRI occured in all except one of the patients who had an abnormal baseline study. In contrast, in most of the patients who had normal baseline perfusion MRI cerebral perfusion remained normal during follow-up.

Compared with conventional MRI, MR angiography (MRA) was the more sensitive neuroimaging modality associated with changes of perfusion. There was a significant association between changes of MRA and perfusion MRI over time. In addition, there were significant correlations between severity of cerebral perfusion abnormality (CBF and MTT) and grade of MRA turbulence both at onset and follow-up. This finding has demonstrated for the first time that there is progression of cerebrovascular disease associated with progression of cerebral perfusion abnormality, which could explain the persistence and severity of the recurrent neurological symptoms despite blood transfusion. Although there was a trend for association between MRA turbulence and the category TCD severity at follow-up (but not in the initial studies), transcranial Doppler ultrasound studies at onset and follow-up also had significant associations with initial and final perfusion MRI parameters (MTT and CBF), showing that TCD could be a useful technique for screening for cerebral perfusion abnormality, although it was as not as sensitive as MRA.

Previous studies using neuroimaging techniques in patients with sickle cell disease demonstrated longitudinal changes in MRI over time from the natural history of the disease where up to 67% of the sickle cell patients who had a first stroke had stroke recurrence without blood transfusion (Powars et al 1978). The prevalence of silent infarcts (an important risk factor for stroke) was reported initially to be 17% between the ages of 6 and 16 years (Moser et al 1996), and then increased to 22% in a recent study of older patients by the same group of collaborators (Pegelow 2002), moreover, another study had reported a prevalence of silent infarcts in SCD up to 23% by 14 years and this proportion increased with age (Miller et al 2001). Longitudinal studies suggest that MRA may predict stroke (Kandeel et al 1996, Seibert et al 1998, Prengler et al 2000). Adams et al showed that using transcranial Doppler ultrasound, critical middle cerebral artery velocities of more than 200 cm/sec were associated with 40% of stroke risk over a period of 3 years (Adams et al 1992 and 1998). Other studies supported the use of TCD in SCD because of its good correlation with MRA abnormality and as a predictor of recurrent neurological symptoms (Siegel et al 1995, Seibert et al 1997, Bernaudin et al 2000).

Studies of cerebral perfusion have been mainly cross-sectional in design. Techniques used previously include xenon inhalation (Huttenlocher 1984, Prohnovik et al 1989, Kugler et al 1993, Tzika et al 1993), positron emission tomography (Herold et al 1986, Powars et al 1999), and techniques of perfusion MRI using either IV gadolinium bolus tracking (dynamic susceptibility contrast MRI, Kirkham et al 2001) or continuous spin-labelling perfusion MRI (Oguz et al 2003), which have demonstrated abnormal perfusion in areas which were normal on T2-weighted MRI. Some of these studies also demonstrated associations between abnormal cerebral perfusion with MRA turbulence and TCD (Venketasubramanian et al 1994, Kirkham et al 2001). Furthermore, there have been a few studies on the beneficial effect of blood transfusion, shortly before and after treatment on cerebral perfusion (Hurlet-Jensen et al 1994, Venketasubramanian et al 1994, Kirkham et al 2001), but the follow-up of those patients was shorter than for the present study study. Another strength of the work presented here is that it has correlated different magnetic resonance techniques longitudinally, focusing mainly on perfusion MRI, instead of only different modalities of neuroimaging.

Most of the patients who had worsening of perfusion MRI, MRI, MRA and TCD had abnormal baseline studies for these investigations, despite being on blood transfusion therapy. Although the statistical analysis of the changes over time of the different modalities did not reach statistical significance among themselves (except for changes over time between MRA and perfusion MRI), there were significant associations between categories of these investigations (such as infarct size and number, MRA turbulence, TCD severity and grades of CBF and MTT) when they were compared at onset and follow-up, and also in relation to the neurological symptoms (at presentation and recurrent), demonstrating related changes over time.

In the case of MRI, infarct number at follow-up was significantly associated with severity of recurrent neurological symptoms, and there were trends for associations between stroke, TIAs, or seizures and worsening of MRI. This finding supports previous studies of recurrent infarction in SCD (Moser et al 1996, Miller et al 2001). Furthermore, the grade of infarct size and number of infarcts were associated significantly with abnormal perfusion parameters (CBF and MTT) either at onset or follow-up.

In relation to MRA turbulence, which is an indicator of cerebrovascular disease, the severity of the MRA turbulence at follow-up was significantly associated with recurrent stroke, TIAs or seizures and with changes of MRA over time, as has been suggested in previous studies (Seibert et al 1998, Prengler et al 2002 and 2003). Additionally, the severity of the MRA turbulence was significantly associated with parameters of abnormal CBF and MTT, which showed the progression of the cerebrovascular disease and its effect on cerebral perfusion and recurrent neurological symptoms without major changes on T2-weighted MRI.

Transcranial Doppler ultrasound has become an important tool for screening cerebrovascular disease in SCD, especially for the detection of vascular disease in the basal arteries of the brain (MCA, ACA). Prevention of stroke in those sickle cell patients who have critical MCA maximum velocities (more than 200 cm/sec) and are therefore at high risk is possible with regular blood transfusion (Adams et al 1992 and 1998). In this study, TCD was shown to be a good technique to detect progression of CVD, but it was not as sensitive as MRA. Categories of severity of TCD were weakly

associated (but not significantly) with MRA turbulence, especially in those studies done at follow-up, and also with severity of the recurrent neurological symptoms. Associations between grade of turbulence and TCD ultrasound pattern, and between TCD and severity of recurrent neurological symptoms, (especially stroke) were reported previously (Siegel et al 1995, Verlhac et al 1997, Seibert et al 1997, Bernaurdin et al 2000). There were also significant associations between TCD patterns of severity and infarct number on the initial and final studies, and with infarct size at follow-up, relationships which have been reported (Adams et al 1988 and 1998, Siegel et al 1995) but not in such extensive studies as reported here.

The association between TCD and perfusion MRI has shown interesting findings not reported before, as previously reports between TCD and cerebral perfusion techniques were based on cross-sectional studies (Kirkham et al 2001) or in relation with blood transfusion therapy (Venketasubramanian et al 1994).

The association between changes of TCD and perfusion MRI over time approached significance. Furthermore, TCD ultrasound grades of severity at onset and follow-up were significantly associated with initial and follow-up grades of abnormal cerebral perfusion (decreased CBF and increased MTT) in patients with SCD. This finding was also seen when comparing initial and final MRA turbulence with initial and final perfusion MRI. Therefore, TCD could be a very practical and non-invasive tool to detect and monitor over time not only cerebrovascular disease in SCD, but also progression of cerebral perfusion abnormality.

In this series, two patients had MCA maximum (maxMCA) velocities more than 200 cm/sec at the onset of this study, and they did not have further overt or covert cerebral infarction during the follow-up period, probably because they were on chronic blood transfusion. One of these patients continued to have persistent maximum MCA velocities of more than 200 cm/sec; however her vascular disease progressed over time and she had decreased velocities > 70 cm/sec in more distal segments of the ipsilateral MCA. Although severely increased velocities >200 cm/sec is a well proven predictor of stroke recurrence (Adams et al 1998), decreased cerebral blood flow velocities could be related to some risk of infarction as well, especially sub-clinical or covert infarct, as has been demonstrated in SCD and in the elderly population (Gilliams et al 1997, Tzourio et

al 2001, Zafeiriou et al 2004). In spite of these data, there are few data on the prediction of new cerebral infarcts or new cerebral atrophy in those sickle cell patients who had decreased blood flow velocities. In the current series, one of ten sickle cell patients (who had maxMCA velocities < 70 cm/sec and ipsilateral MCA lowest: highest ratio ≤ 0.5) developed a new infarct and 2 other patients had progression of focal cerebral atrophy, despite blood transfusion. In fact, TCD patterns of severity (where decreased maxMCA velocities <70 cm/sec is worse than maxMCA velocities > 200 cm/sec, because it is related to lower arterial blood flow secondary to very severe artery stenosis) were significantly associated with infarct number and size at follow-up, which suggests that those sickle cell patients with low TCD blood flow velocities (with a demonstrated ultrasound window) could be considered at risk of further cerebral infarction or progression of cerebral atrophy, especially if they have concomitant severe recurrent neurological symptoms such as TIAs and seizures as an expression of CVD and cerebral ischaemia (Kirkham et al 2001, Prengler et al 2002 and 2003).

The limitations of the technique of the transcranial Doppler ultrasound depend on the experience of the operator who performs the study, and the ultrasound window which can vary with age and individual (e.g. thick skull secondary to bone marrow changes). This very useful screening tool could give false positives (decreased MCA blood flow or undetectable MCA) in the presence of an inadequate ultrasound window (i.e. thick skull), and the TCD study should be evaluated with caution in the context of the patient's symptomatology and neuroimaging. As was shown in this study, there were initially 7 patients with normal MRA, and only 2 of them had normal TCD following Adams's criteria (Adams et al 1992). However, TCD might be a more sensitive to detect flow turbulence (and probable cerebrovascular disease) than MRA in sickle cell disease. Therefore, evidence-based guidelines for acceptable depths for the ultrasound window according to age of the patient and conditions which might affect skull thickness (as seen in SCD) should be addressed in further studies.

One quarter of the sickle cell patients (6/23) had an improved perfusion MRI study at follow-up; all had had an abnormal baseline study and they were, at least initially, on chronic blood transfusion therapy. Nevertheless, an improvement in their cerebral perfusion was not accompanied with an improvement of their recurrent neurological symptoms, as these patients had TIAs, seizures and headaches. However, a smaller

number of patients had improved MRA (4/23 [13%]) and TCD (2/23 [8%]). Including all recurrent neurological symptoms, there does not seem to be an association with cerebral perfusion. In fact, these patients did not have stroke or coma [PLKE] recurrence, and probably the absence of these two very severe recurrent symptoms can be the real indicators of unchanged or improved cerebral perfusion. Transient ischaemic attack was a symptom found in patients with worse, unchanged, and improved investigations. TIAs were found, as well, in the same proportions in patients with improved or worse studies. However, this group of patients confirms that blood transfusion therapy could help to improved cerebral blood flow and vascular disease as has been shown in previous studies but with a shorter follow-up period (Hurlet-Jensen et al 1994, Venketasubramanian et al 1994, Kirkham et al 2001), although this improvement was seen in a smaller proportion of patients in comparison with those with unchanged or worse studies.

More than a third of the patients had unchanged MRI perfusion, and the majority also had normal baseline perfusion studies; recurrent headache was the most frequent symptom and was significantly associated with unchanged perfusion MRI at follow-up. Only one patient of 7 with a normal baseline perfusion MRI was on chronic blood transfusion, with one other on Hydroxyurea, whereas those patients with unchanged perfusion MRI who had had abnormal baseline studies were on blood transfusion therapy.

Most of the patients with unchanged normal perfusion MRI also had normal MRI and MRA studies at onset and at follow-up. TCD was the least correlated study for this group of patients. There was initially a small number of patients with normal TCD, perhaps because of technical difficulties in detecting the MCAs due to the ultrasound window, which could change depending on age and individual (see limitations of TCD explained in chapter 3 and above). This group of patients showed that most of the patients with normal baseline studies and less severe recurrent symptoms maintained normal cerebral perfusion and neuroimaging over time. In a few additional patients, blood transfusion may have helped to stabilise the pathology documented using magnetic resonance techniques.

However, comparing MRI, MRA, TCD and perfusion MRI, it is interesting that of patients on chronic blood transfusion who had abnormal baseline studies, only 2 patients had unchanged perfusion MRI, whereas there were higher numbers for MRI (n=8), MRA (n=6) and TCD (n=6) with unchanged studies. However the conventional neuroimaging and TCD could not detect particularly improvements of cerebral perfusion (6 patients) specifically compared with MRA (n=2), or to a lesser degree worsening of cerebral perfusion (n=7), especially against MRI (n=5) if we compared equal numbers of patients (for TCD the sample was smaller). Therefore, perfusion MRI is a very sensitive investigation for the evaluation of changes over time.

Regarding perfusion MRI parameters using dynamic susceptibility contrast MRI (DSC-MRI), cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) are very sensitive indicators for identifying areas of cerebral tissue which are risk of ischaemia (Calamante et al 1999, Gadian et al 2000). However, a limitation of this study is that the assessment of cerebral perfusion abnormality is qualitative (visual inspection); although quantitation would provide a measure of real changes in cerebral blood flow, this is not technically possible using this technique at present. Visual inspection entails a degree of subjectivity regarding the extension and severity of the regional cerebral perfusion abnormality.

The main perfusion MRI parameters taken for analysis with the other modalities of investigation were cerebral blood flow (decreased [more frequently seen] or increased) and mean transit time (increased). These parameters are usually inversely related; a decrease in CBF is associated with an increase in MTT. The increased MTT of the passage of the intravenous bolus of Gadolinium occurs because there is slow passage of blood secondary to cerebrovascular disease (large or small blood vessel disease [Calamante et al 1999, Pavlakis et al 1988 and 1989, Prengler et al 2002]). Decreased or unchanged CBF has been shown to be a more sensitive parameter of cerebral perfusion abnormality than MTT in relation to clinical symptoms, neuroimaging and TCD in patients with SCD. Although most of the MR studies and TCD were significant for both parameters, CBF reached the highest level of significance when compared with MTT. This may be because increased MTT appears highlighted more on visual inspection than CBF (MTT is white and CBF is dark on the perfusion maps), and therefore for the observer it is more difficult to assess different grades of severity in the MTT maps than

in the CBF maps. Because modifications in CBF have to be fairly marked in order to detect the differences over time on the CBF maps on visual inspection, it is possible that they were more correlated with the real changes (and with the other investigations) than MTT. In fact, for the analysis of the perfusion data over time, changes were recorded only when there were at least two severity grades of difference between the initial and final studies for both parameters CBF and MTT, to increase the robustness of the findings by minimising the effects of differences between independent observers.

In this series, there were some patients who had decreased CBF (usually in association with decreased CBV) with normal MTT initially and at follow-up. The decrease of CBF alone could be an early indicator of tissue at risk of ischaemia (that usually is well demonstrated with increased MTT). Conversely, there was only one patient in this series (described as a case report in a section above) who had persisting increased CBF (and CBV) initially with normal MTT and increased CBF (and CBV) during follow-up. This increase in CBF and CBV could be secondary to hyperaemia or vasodilation (Prohovnik et al 1989); however some regions with initial increased CBF progressed to decreased CBF and increased MTT in the final perfusion MRI. Therefore, it could be suggested that either isolated increased CBF or decreased CBF could be both indicators of further risk of regional cerebral ischaemia.

New perfusion MRI techniques with quantification of the CBF could help to define areas of decreased cerebral perfusion more accurately. A preliminary study in children with SCD has been published recently using continuous arterial spin- labelling (ASL) perfusion MRI, demonstrating that there were areas of decreased CBF (quantitative measurement), which were related to the symptoms of the patients (cognitive deficits) with normal conventional MRI scans (Oguz et al 2003). Currently, this technique is constrained by signal-to-noise limitations, which has prevented more widespread clinical application; nevertheless, research continues in this area of magnetic resonance, and it is likely to become more widely applied in the near future. It should also be noted that the assertion that the values obtained by ASL are truly quantitative is as yet controversial, since this technique also has a number of technical limitations. At the moment DSC-MRI with intravenous bolus of Gadolinium as been the most widely applied in clinical applications, and has shown to be a sensitive technique for diagnosis and monitoring sickle cell patients with neurological symptoms.

Although many of these patients were on blood transfusion or other alternative treatments there was progression of cerebrovascular disease during the follow-up period of this study. The sickle cell can damage the endothelium not only due to its abnormal shape but also because it triggers a cascade of molecular factors and blood components that lead to the pathogenesis of cerebrovascular disease, and this process continues over time (Pavlakis et al 1989, Stemberg 1999, Belcher et al 2000, Franco et al 2000, Prengler et al 2002). The aim of blood transfusion is try to reverse this process (Pavlakis et al 1989, Ballas 1998); however, it was demonstrated in this series that the progression of cerebrovascular disease continued in 26% of the patients and remained unchanged in another 26%, while there was not a complete reversal of the CVD.

Other clinical parameters which could affect blood vessels are blood pressure and haematological factors, and these were studied in these sickle cell patients in relation to the magnetic resonance investigations and TCD.

The effects of hypertension on the cerebral vasculature, which leads to vascular changes and is an important risk factor for stroke in the adult population, have been well documented. 'Relative' hypertension has been reported in sickle cell patients as associated with an increased risk of stroke and mortality (Rodgers et al 1993, Pegelow et al 1997). Increased blood pressure was also considered a risk factor for stroke in the Cooperative Study of Sickle Cell Disease (Ohene-Frempong et al 1998). In the series described in this thesis there were no significant associations between systolic, diastolic and mean arterial blood pressures at follow-up and worsening of MRI, MRA, TCD and perfusion MRI, although systolic blood pressures were above the 50^{th} percentile for age but not beyond the 90^{th} percentile. Only one patient had a hypertensive crisis in the context of a posterior leukoencephalopathy, and he had a new cerebral infarction at follow-up on his final MRI scan. Perhaps, because of the small numbers, any effect of blood pressure did not reach statistical significance, although there was a trend for association between low diastolic pressure and worsening of TCD over time, which could contribute to the decrease of the blood flow velocities.

Regarding haematological factors, there was a significant association between decreased initial platelet count and worsening of MRA. In addition, worsening of cerebral

perfusion and worsening of MRI over time were related to increased white cells and lymphocytes at follow-up. There were no significant associations for haemoglobin levels. Blood components related to infection (i.e. white cells and neutrophils) have been related to the pathogenesis of CVD in sickle cell disease, and form part of the cascade of factors triggered either by the sickle cell itself or sepsis; this cascade also involves platelet activation (Sultana et al 1998, Belcher et al 2000, Inwald et al 2000). Sickle cell patients suffered from frequent infections, either bacterial or viral, from early years of life (Ballas et al 1998). This study showed that there are trends for associations (the numbers of patients with haematological data were too small) between infection and perfusion abnormality and MRI, and between thrombocytopenia and MRA (which can be related to chronic infection). A preliminary study presented in abstract form showed progression of CVD in sickle cell patients who had stroke with increased lymphocyte counts in comparison with non-sickle children with stroke (Prengler et al 2000). Reducing the infection rate in these patients with environmental and medical measures (although they are already receiving prophylactic vaccines and Penicillin) may help to reduce the progression of CVD, especially in those children who have already had or are at risk of stroke. The effect of blood transfusion on the cerebral vasculature secondary to iron overload and its contribution to the progression of the CVD in SCD could be investigated in further studies.

Patients' follow-up daytime awake oxygen saturations were not significantly associated with worsening of perfusion, MRI, MRA and TCD. Daytime awake-oxygen saturations were 92% or more, above the cut-off for hypoxemia (less than 92%). However, these normal awake-oxygen saturations did not exclude the possibility that these patients had nocturnal hypoxemia and obstructive sleep apnoea that have been associated with central nervous system events in SCD (Davies et al 1989, Kirkham et al 2001).

Finally, another aspect of this longitudinal study was to identify predictors of recurrent clinical symptoms and worsening of magnetic resonance studies. For worsening of cerebral perfusion at follow-up there were several significant associations, such as severity of onset and recurrent neurological symptoms, initial infarct size and number, grade of initial MRA turbulence and initial grade of decreased CBF or increased MTT. These showed that an important number of these patients maintained their initial symptoms and parameters throughout time without significant changes with or without

treatment. A correlation matrix showed that there was a good correlation among initial infarct size, infarct number, CBF and grade of MRA turbulence. As a consequence, there appear to be many significant predictors in relation to worsening of perfusion MRI, in part because these predictors are significantly correlated among themselves. That means that, in this preliminary study, cerebral perfusion at follow-up could be predicted with one or two MR studies in addition to the initial or recurrent symptoms of the patient, while a number of the modalities of investigations provide only corraboration of these findings, without additional independent information.

Prediction of recurrent neurological symptoms, MRI and MRA changes at follow-up have been investigated previously (Ohene- Frempong et al 1998, Moser et al 1996, Miller et al 2001, Pegelow et al 2002, Seibert et al 1998). However, a number of additional variables different variables were analysed in the work described in this thesis.

Potential predictors of the severity of the recurrent neurological symptoms were severity of the symptoms at onset, initial number of infarcts, and grade of MRA turbulence. Predictors of recurrent cerebral infarction (infarct size and number on final MRI), were initial infarct size and number, initial MRA grade of turbulence and initial abnormal grade of CBF. Initial TCD ultrasound pattern was significantly associated with final infarct number. And finally, predictors of MRA turbulence at follow-up were severity of the initial neurological symptoms, initial infarct size and number, severity of decreased CBF or increased MTT on the initial perfusion MRI, and initial grade of MRA turbulence. However initial TCD was not a significant predictor for MRA turbulence at follow-up. A number of these significant associations were correlated among themselves.

However, these predictors showed that patients who had severe neurological symptoms at onset are more prone to have severe recurrent neurological symptoms at follow-up, with the exception of stroke recurrence, which might be prevented by the chronic blood transfusion or other alternative therapies. In addition, these predictors demonstrated that there are no substantial changes in the severity of MRI and MRA over time in most of the patients, with an exception of perfusion MRI, which showed improvement and

worsening of the cerebral perfusion in a higher proportion of patients than the other techniques.

Patients who had areas of perfusion abnormality maintained or worsened their grade of perfusion abnormality over time, and their perfusion MRI studies were unchanged or worse at follow-up. Nevertheless, those patients who improved their abnormal perfusion at follow-up, still had areas of persisting perfusion abnormality although in their final perfusion MRI, they had specific regional improvements of their cerebral perfusion.

In practice, the worst grades of CBF and MTT were chosen for statistical analysis, independently of whether the perfusion MRI had improved, remained unchanged or worsened. Because those patients whose cerebral perfusion improved still maintained the same initial grades of perfusion abnormality in some areas of the brain at follow-up, their initial and final CBF and MTT had a significant association with the other magnetic resonance studies and TCD (which maintained or worsened their grades of severity over time in a great proportion of the sickle cell patients). The same principle applies to MRA turbulence; while some patients had an improvement of the MRA turbulence, the severity of the CVD usually persisted over time.

In summary, this study demonstrated that there is not a complete reversal of CVD and perfusion abnormality in the majority of the patients who had abnormal baseline studies, and that the initial grade of severity usually persisted or impaired over time despite different therapies.

5.11. Conclusion

In conclusion, this study showed that there was progression of cerebral perfusion abnormality over time in more than one third of the patients with sickle cell disease. The progression of abnormal perfusion was correlated with the severity of the recurrent neurological symptoms and with the progression of cerebrovascular disease (MRA turbulence) and it continued despite chronic blood transfusion therapy or other

alternatives therapies. In addition, more than a third of patients had unchanged cerebral perfusion, MRI and MRA during the follow-up.

There was a good correlation between transcranial Doppler ultrasound and MRA at follow-up and between TCD and perfusion MRI. TCD could be a useful tool, not only to monitor progression of CVD but also progression of perfusion abnormality over time. The progression of cerebral perfusion abnormality and cerebrovascular disease might be associated with infection in addition to the damaging effect of the sickle cell in the vascular endothelium.

Perfusion MRI seems to be the most sensitive technique to evaluate changes over time, and was often associated with recurrent symptoms in this population. Changes over time of perfusion MRI, structural MRI, MRA and TCD, and predictors of cerebral perfusion abnormality at follow-up, showed that there is rarely a complete reversal of the cerebrovascular disease, and the perfusion abnormality in those sickle cell patients who had abnormal baseline studies and the initial grade of severity usually persisted or worsened over time despite different therapies.

Chapter 6: Effect of Blood Transfusion Therapy on Perfusion Abnormality in the Short and Long Term in Sickle Cell Disease

6.1. Introduction and Aims

6.1.1 Introduction

Long-term blood transfusion is recommended for the prevention of recurrent stroke in sickle cell disease (Pavlakis et al 1989, Ballas et al 2002, Kirkham and deBaun 2004). Without blood transfusion, recurrent stroke occurs in up 67% of patients with SCD (Powars et al 1978). However, in sickle cell patients on blood transfusion stroke recurs in up to 13%, although keeping the HbS% less than 30% in patients on chronic transfusion is important in reducing the recurrence risk (Pegelow et al 1995). Reported secondary stroke rates have been from 2.2. to 6.4 events per 100 patient-years in sickle cell patients on blood transfusion after their first stroke (Pegelow et al 1995, Scothorn et al 2002). Worryingly, Dobson demonstrated that up 41% of SCD patients experienced recurrence of central nervous system (CNS) events despite chronic transfusion, in association with a high prevalence of cerebrovascular disease (particularly moyamoya syndrome, with occlusion of the distal internal carotid artery and collaterals, in 43%) in their series (Dobson et al 2002).

There have been few studies, which have mainly used inhaled $Xenon^{133}$, investigating the effects of blood transfusion on cerebral blood flow (CBF) before and after blood transfusion in patients with SCD. These studies demonstrated the beneficial effect of blood transfusion in normalising CBF (Prohovnik et al 1989, Hurlet-Jensen et al 1994) or reversing the abnormal regional perfusion in patients with acute neurological complications such as stroke, TIA, coma or seizures (Huttenlocher et al 1984). A recent cross-sectional study in patients with SCD and neurological complications (using perfusion MRI using intravenous bolus of Gadolinium [DSC-MRI]) showed an

improvement of the regional perfusion abnormality in one patient, shortly after blood transfusion (Kirkham et al 2001).

There is increasing interest in treating children and adults with SCD with alternative therapies, such as Hydroxyurea (Ware et al 1995, Ware et al 1999, Ware et al 2004) and bone marrow transplantion (Walters et al 2000). Pilot trials of children with SCD and stroke treated with Hydroxyurea reported secondary stroke rates of 5.7 and 3.6 events per 100-patient-years (20% and 10% of the patients respectively) over a follow-up period of up to 42 months in children who received only Hydroxyurea and in those who received Hydroxyurea overlapping with blood transfusion respectively (Ware et al 2004), a similar incidence to those treated with regular blood transfusion.

However, chronic blood transfusion currently remains the mainstay of treatment for SCD patients who have stroke or severe neurological complications. There have been no studies on the effect of long-term blood transfusion on cerebral perfusion and cerebrovascular disease, which could provide valuable information, as there are concerns about the side effects of chronic blood transfusion (i.e. iron overload, blood borne infections, erythrocyte autoantibody formation [Ballas et al 2002]) and poor compliance with this treatment by a significant proportion of the patients.

6.1.2. Aims

The primary aim of this study was to examine the effect of blood transfusion on cerebral perfusion (imaged using magnetic resonance bolus tracking of Gadolinium) and cerebrovascular disease (determined using magnetic resonance angiography and transcranial Doppler) in both the short-term (pre- and post-blood transfusion) and in the long-term (post-chronic transfusion) in patients with sickle cell disease and central nervous system events.

Although there would be additional interest in considering the effects of other alternative treatments used in SCD (i.e. Hydroxyurea, bone marrow transplant and revascularisation procedures) on cerebral perfusion and cerebrovascular disease, especially in those patients who had stopped blood transfusion and continued on another

therapy, these data were only available in three patients, and are therefore included as cases reports only.

6.2. Subjects

6.2.1. Short-Term Blood transfusion

Data collection for the patients of this study was described in chapter 2 (Appendix-Table 1). Sickle cell patients with CNS events (stroke or TIA) on blood transfusion therapy (BTx) were recruited from referrals to the joint Haematology/Neurology clinics attended by MP and FK at five North London hospitals (Central Middlesex, North Middlesex, University College (UCH), Royal London and Whittington Hospitals).

Nine patients with sickle cell anaemia (HbSS) who had successful magnetic resonance studies were originally selected for this series. One of the nine patients (patient No. 15, Appendix-Table 1) had successful perfusion MRI studies, but was excluded from this series because pre- and post-blood transfusion MR perfusion scans were in different positions and orientations, therefore it was difficult to conclude whether there had been significant changes in perfusion abnormality between the studies.

The final series consisted of eight patients with sickle cell anaemia, 4 were male and the mean age was 16.3 years (range 8 to 27 years).

6.2.2. Long- Term Blood transfusion

Data collection for the patients of this study was described in chapter 2 (Appendix-Table 1). Sickle cell patients with CNS events on chronic blood transfusion therapy were recruited from referrals to the joint Haematology/Neurology clinics attended by MP and FK at six North London hospitals (Central Middlesex, North Middlesex, University College (UCH), Royal London, Whittington and Saint Mary's Hospitals).

Seventeen patients with sickle cell anaemia (HbSS) had successful initial and follow-up magnetic resonance studies. These patients were part of the longitudinal study described

in chapter 5 (17/23 patients). Six of the 17 patients were included in the short-term blood transfusion study described above. Seven patients were male; the mean age at the time of their first MR studies was 12.9 years (range 6.8 to 21.9 years) and the mean age at the follow-up MR studies was 15.2 years (range 7.5 to 23.9 years).

6.3. Methods

6.3.1. Data Acquisistion

The methods for the acquisition of conventional neuroimaging, perfusion MRI, transcranial Doppler ultrasound, oxygen saturation, blood pressure measurements and haematological parameters have already been described in chapter 3.

For the short-term blood transfusion study, the patients (n=8) underwent magnetic resonance (MRI, DWI, MRA and perfusion MRI); transcranial Doppler ultrasound investigations (pre-BTx n=8; post-BTx n=8); blood pressure measurements, which included systolic, diastolic and mean arterial blood pressures (pre-BTx=7; post-BTxn=6); and 3 minutes' awake-pulse oximetry [SpO_2] (pre-BTx= 7; post-BTxn=7); with a mean of 3.9 days (range 2 to 7 days) before blood transfusion and a mean of 4.7 days (range 0.2 to 12 days) after transfusion. Haematological parameters were collected from clinical records, only haemoglobin was used for analysis of this study. HbS% and ferritin data were also collected but the numbers were too small for analysis and therefore they were excluded from this study; this study did not included analysis of white cell and platelet counts.

For the long-term blood transfusion study, 17 patients underwent initial and follow-up magnetic resonance studies, and initial (n=13) and follow-up (n=17) transcranial Doppler ultrasound. The follow-up investigations occurred at a mean of 2.2 years (range 8 months to 3.5 years) after the initial investigations. Initial blood pressure measurements (n=2) and haematological parameters were collected from clinical records, however only haemoglobin was used for analysis as described above; follow-up blood pressure measurements were recorded at the same day of the magnetic resonance studies in 13 patients. Analysis of the oxygen saturation (SpO_2) of the

patients was not included in the long-term blood transfusion study because there were no initial SpO_2 data.

6.3.2. Data Analysis

Wilcoxon test were used for comparison of non-parametric continuous data (blood pressure and haemaglobin levels). Level of significance was defined as $p<0.05$, and a trend for significance was set between $p \geq 0.05$ and $p \leq 0.1$.

6.4. Results I: Short-Term Blood Transfusion

6.4.1. Effect of Short-Term Blood Transfusion on Neurological Symptoms

The sickle cell patients on blood transfusion therapy had had the following central nervous system (CNS) events at presentation (Appendix-Table 5): stroke in 6 patients (one of the stroke patients presented with coma); anterior territory transient ischaemic (TIA) attacks in one; and posterior territory TIA (collapse and transient loss of vision) in one.

Of the sickle cell patients who had had stroke at onset, the majority had a history of recurrent neurological symptoms such as anterior territory TIAs in 2; headaches in 3; one patient remained asymptomatic. The patient who had initially anterior territory TIA became asymptomatic; and the other who presented with posterior territory TIA had recurrent headaches.

Shortly after blood transfusion, all of these patients experienced an improvement in their general symptoms caused by SCD; in 6 out of 8 patients the recurrent neurological symptoms diminished in frequency (n=4); 2 remained asymptomatic. Two patients continued having headaches (1 with stroke and another with posterior TIA at onset); however their generalised symptoms improved after transfusion.

6.4.2. Effect of Short-Term Blood Transfusion on Blood Pressure

The mean of the systolic blood pressure (SBP) of the patients (n=7) before transfusion was 106 mmHg (range 85-121 mmHg); the diastolic blood pressure (DBP) was 52 mmHg (range 31-75 mmHg); and the mean arterial blood pressure (MAP) was 71 mmHg (range 61-90 mmHg).

After blood transfusion (n=6), SBP mean was 113 mmHg (range102-121 mmHg); DBP was 60 mmHg (range52-73 mmHg); and MAP was 77 mmHg (range 68-89 mmHg).

There were trends for associations between pre- and post-blood transfusion blood presssure measurements for a higher SBP (p=0.08, Wilcoxon test) and a higher MAP (p=0.1; Wilcoxon test) after transfusion. There was no association for DBP (p=0.3) before and after transfusion.

6.4.3. Effect of Short-Term Blood Transfusion on Oxygen Saturation

Pre-blood transfusion, the sickle cell patients had a mean awake-Oxygen saturation (SpO_2) of 96.5 % (range 92 to 98.4%). Post-blood transfusion the awake-SpO_2 mean was 97% (range 94-98.9%). Awake-SpO_2 was significantly higher after blood transfusion (p=0.028; Wilcoxon test).

6.4.4. Haemoglobin

Available haemoglobin data were collected from 5 patients. The blood tests were done at a mean 4 days (range 0-1 months) from the neuroimaging and TCD studies. Haemoglobin levels pre- and post- transfusion were not available in the clinical records for all patients. The mean haemoglobin level was 10 g/dL (range 9.2 to 10.9 g/dL).

6.4.5. Effect of Short-Term Blood Transfusion on Magnetic Resonance Imaging

Appendix-Table 5 shows the relationship between the results from different MR investigations and transcranial Doppler ultrasound (TCD) in the patients included in this study. Of the 6 patients who presented with stroke at onset, 1/6 had an unilateral

cerebral infarct in the anterior cerebral artery (ACA)/ middle cerebral artery (MCA) territories on T2-weighted MRI; the remaining 5 patients had bilateral infarcts, in the ACA/MCA territory in 3 patients (1/3 with cerebral atrophy), and in the posterior cerebral artery (PCA)/MCA territories in 2 patients (both had cerebral atrophy).

The sickle cell patient who had anterior territory TIAs had unilateral deep watershed white matter infarcts and the patient who presented with posterior territory TIAs both had bilateral deep watershed infarcts in the MCA/ACA territories.

None of the patients had any change on T2-weighted MRI after short-term blood transfusion.

6.4.6. Effect of Short-Term Blood Transfusion on Magnetic Resonance Angiography

The maximum grade of turbulence for each patient is described in Appendix Table 5. Before blood transfusion 1 patient had a normal and 7 had abnormal MRA studies.

Of the six patients who had stroke at onset, 3 patients presented with artery occlusion (ACA-A1 [n=2]; MCA-M1 [n=1, plus moyamoya collaterals]); 1 had moderate turbulence (terminal internal carotid artery [TICA]/MCA/ACA) and 2 patients had severe turbulence on MRA (TICA, n=1; MCA-M1, n=1). The patient who had initially anterior territory TIAs had a normal MRA, and the other with posterior territory TIAs had mild turbulence on MRA (ACA-A1).

After blood transfusion, MRA was unchanged in 4 patients (2 stroke patients with artery occlusion, 1 stroke patient with moderate turbulence and a patient with posterior TIAs with previously mild turbulence). MRA improved bilaterally in one patient with stroke (the severe turbulence in the TICA remained but other segments normalised from mild to moderate turbulence i.e. MCA-M2, ACA-A1 and PCA-P1). Three patients had a worse MRA after blood transfusion unilaterally; one stroke with artery occlusion (MCA) and moyamoya collaterals (mild turbulence to artery occlusion of the PCAs), another stroke patient with initial severe turbulence on MRA on the TICA (this segment remained unchanged but other MCA/ACA segments progressed to moderate and severe

turbulence), and the patient who had had anterior TIAs and normal initial MRA, who had mild turbulence in the MCA after transfusion.

In summary, 4/8 of the patients had unchanged, 3/8 worse (unilaterally), and 1/8 had an improved (bilaterally) MRA studies shortly after blood transfusion.

6.4.7. Effect of Short-Term Blood Transfusion on Perfusion MRI (DSC-MRI)

Appendix-Table 5 and Appendix-Table 6 present the regions of cerebral perfusion abnormalities on DSC-MRI and changes of cerebral perfusion after blood transfusion. Initial perfusion MRI was normal in 1 and abnormal in 7 of these 8 sickle cell patients.

Before blood transfusion, one patient had a normal perfusion study (anterior territory TIA), 2 stroke patients had unilateral abnormal perfusion confined to a region or regions of a cerebral hemisphere (MCA/ACA territories); and 5 patients (4 stroke; 1 posterior TIAs) had bilateral perfusion abnormalities in the ACA/MCA territories (n=3) and in the MCA/PCA territories (n=2).

After blood transfusion, 3 patients had unchanged perfusion MRI; in one patient with a previous normal study, and in 2 stroke patients who had had bilateral perfusion abnormalities in the ACA/MCA (n=1) and MCA/PCA (n=1) territories.

However, five patients showed improved perfusion after transfusion in at least one cerebral region; the improvement in cerebral perfusion abnormality was characterised by a decrease in the mean transit time (MTT; n=2); an increase in the cerebral blood flow and cerebral blood volume (CBF and CBV; n=1), an increase in CBV only (n=1) and a decrease in MTT with an increase in CBF and CBV (n=1). One patient (posterior TIA) with previous bilateral abnormal perfusion improved in regions of one hemisphere (ACA/MCA). For the 4 remaining patients (stroke), 2 patients who had had initial bilateral abnormal perfusion improved in both hemispheres in the MCA/ACA (n=1) and PCA/MCA (n=1) territories; and two patients with previous unilateral perfusion improved in some regions of the MCA territory (n=1) and in a localised region of the ACA territory (n=1, pat. 2, Appendix-Table 5). However, there was not a complete reversal of the perfusion abnormality for any of these patients after blood transfusion.

In summary, 3 of 8 sickle cell patients had unchanged perfusion MRI studies and 5 out of 8 had a partial improvement (either unilateral or bilaterally) of their perfusion abnormality shortly after blood transfusion.

6.4.8. Effect of Short-Term Blood Transfusion on Transcranial Doppler Ultrasound

Before blood transfusion, one patient had a normal TCD (stroke; normal MCA velocities, but decreased PCA velocities) and 7 patients had abnormal TCD, according to Adams' criteria (Adams et al 1992 and 1998).

Of the patients who had initially abnormal TCD, 5 patients (3 stroke, 1 anterior and 1 posterior territory TIAs) had mean maximum MCA (maxMCA) velocities less than 70 cm/sec and lowest: highest ipsilateral MCA ratio <0.5; 2 of the stroke patients had undetectable MCA.

Shortly after blood transfusion 6 patients had unchanged TCD studies including the patient with previously normal TCD, and there was an improvement in 2 patients who normalised their TCDs in one MCA (1 posterior territory TIA and 1 stroke).

In summary, 6/8 of the patients had unchanged and 2/8 had improved TCD shortly after blood transfusion (figures 6.1 and 6.2). Table 6.1 shows the relationship between the different studies before and after blood transfusion in this series of sickle cell patients.

Investigations	Better Post-BTx		Unchanged Post-BTx		Worse Post-BTx	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
MRI (n=8)				**8**		
MRA (n=8)		**1**		**4**	**1**	**2**
TCD (n=8)		**2**	**1**	**5**		
Perfusion MRI (n=8)		**5**	**1**	**2**		

Table 6.1. Relationship between results of the different investigations after blood transfusion in this series of sickle cell patients. BTx= blood transfusion.

No data because no improvement can be demonstrated from a normal study or from a study showing the presence of lesions.

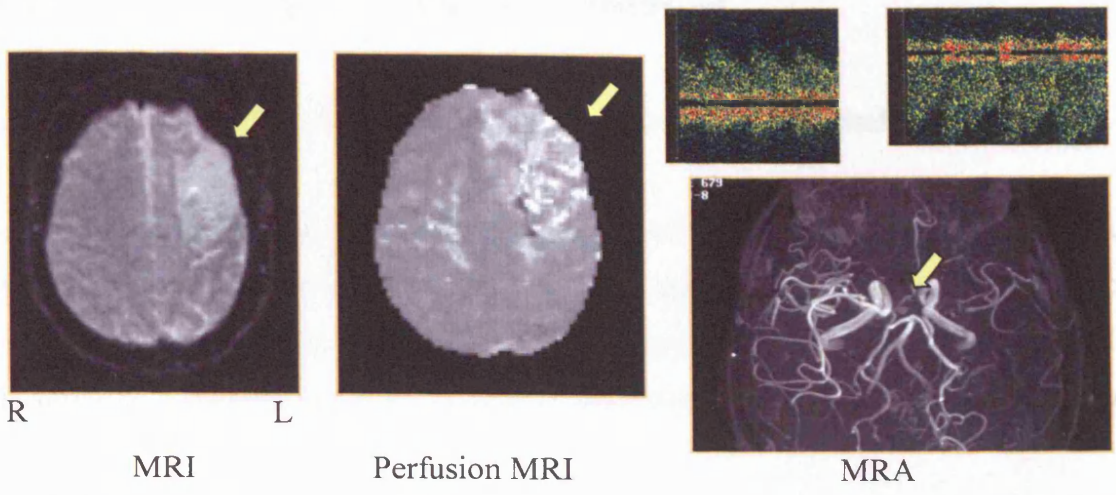


Figure 6.1. Short-term blood transfusion. *Pre-Blood transfusion*. 15-year old female, SCD (HbSS), stroke at 12 years of age. Recurrent headaches. ***T2-w MRI***. Mature left middle cerebral artery (MCA) infarct, left caudate nucleus infarct and atrophy. ***Perfusion MRI***: Abnormal perfusion MRI. Increased mean transit time of the passage of IV contrast bolus in the left MCA territory, including the left frontal region. ***MRA***. Stenosis of the left MCA and Left A1 (anterior cerebral artery), collateral vessels. ***TCD:*** decreased left MCA blood flow velocities and turbulent left ACA flow. R: right; L: left.

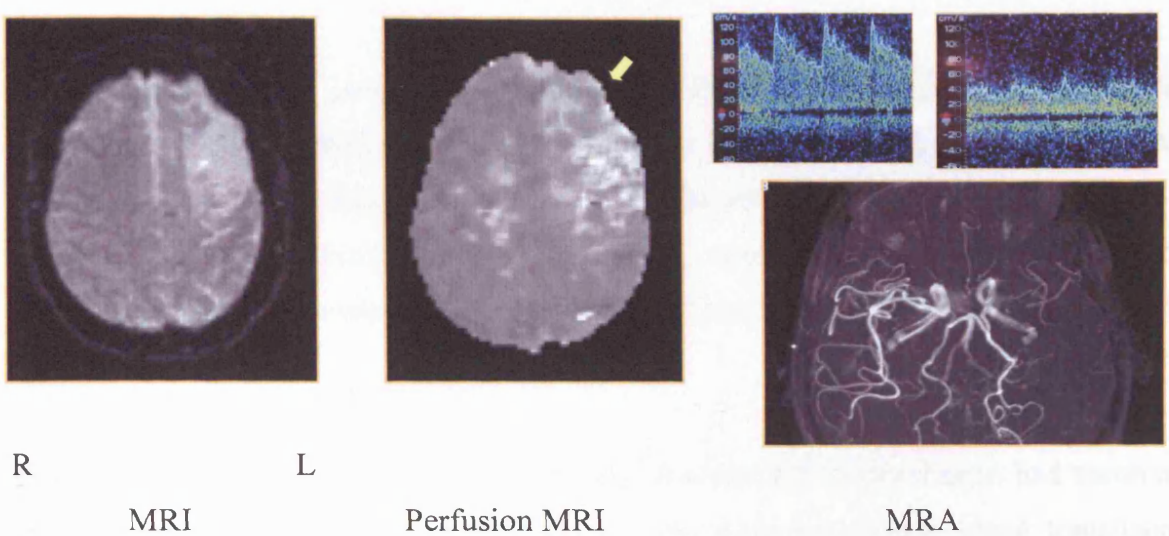


Figure 6.2. Short- term blood transfusion (same patient). *Post-Blood transfusion (six days post-transfusion)*. ***T2-w MRI***. Unchanged MRI. Improved ***Perfusion MRI***.Decreased (compared to pre-transfusion) mean transit time of the passage of IV contrast bolus (and increased cerebral blood flow) in the left frontal region and, in some degree, in the left MCA territory. ***MRA***. Unchanged for the left MCA & ACA. Slight improvement in the visibility of distal vessels. ***TCD***: slight increased of the left MCA blood flow velocities and less turbulent flow in the left ACA. R: right; L: left.

6.5. Results II: Long-Term Blood Transfusion

6.5.1. Long-Term Blood Transfusion and Recurrent Neurological Symptoms

The 17 sickle cell patients involved in this study had had the following central nervous system events at presentation (Appendix-table 3 [patients 1- 16 and 20] and Appendix-Table 7): coma with stroke in 2 out of the 17 patients; coma and posterior territory TIA in 1; stroke in 7; anterior territory TIAs in 4; posterior territory TIAs in 2 and headaches in 1.

All 17 patients were initially on blood transfusion therapy; however during follow-up, only 9 out of the 17 remained on chronic blood transfusion, whereas 5 patients stopped blood transfusion (one due to autoantibody formation; patient 8, Appendix- table 3); 1 patient stopped and continued on Hydroxyurea; 1 patient stopped transfusion and received a bone marrow transplant; and another stopped transfusion and had subsequent surgical revascularisation and continued on Hydroxyurea.

During the follow-up period, the patients had the following recurrent neurological symptoms: 1 patient had coma (with posterior leukoencephalopathy on MRI [this patient appears in the figures as 'PLKE']); 1 had stroke; 6 headaches; 1 seizures; 6 anterior and 1 posterior territory TIAs; and one remained asymptomatic. The relationship between recurrent neurological symptoms and therapies are shown in table 6.2 and figure 6.3.

From table 6.2 and figure 6.3, a patient who stopped blood transfusion had recurrent stroke, whereas all but one of the patients who were on chronic blood transfusion continued to have recurrent neurological symptoms. Patients who had stopped blood transfusion or who were on alternative treatments for SCD, also presented with recurrent symptoms, in the majority of the cases, in relation to their baseline CNS event at onset (Appendix - table 3).

Recurrent Symptom (n=Pats)	BTx	BTx Stopped	BTx Stopped HU	BTx Stopped BMT	BTx Stopped RV+HU
None	1				
Headache	3	2	1		
Seizures	1				
Post. TIA		1			
Ant TIA	3	1		1	1
Stroke		1			
Coma (PLKE)	1				
Total	9	5	1	1	1

Table 6.2. Recurrent neurological symptoms in patients with sickle cell disease and their different therapies. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure; PLKE= posterior leukoencephalopathy

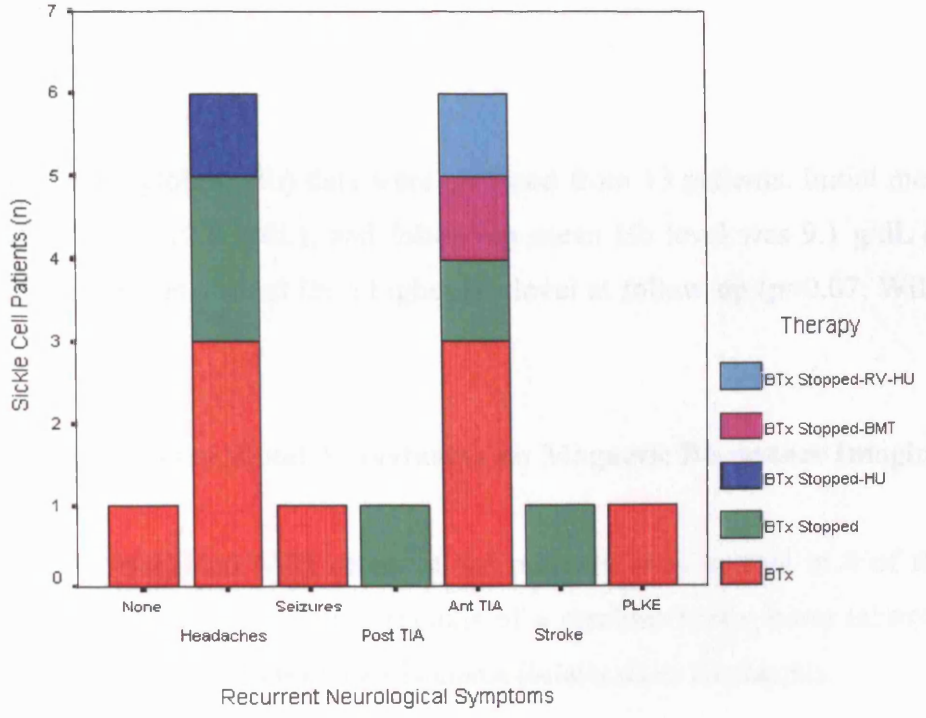


Figure 6.3. Recurrent neurological symptoms in patients with sickle cell disease and their different therapies. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure; PLKE= posterior leukoencephalopathy

6.5.2. Blood Pressure Measurements

The mean of the systolic blood pressure (SBP) of the patients (n=2) at the time of the initial investigations was 120 mmHg (range 120-120 mmHg); the mean diastolic blood pressure (DBP) was 55 mmHg (range 50-60 mmHg); and the mean arterial blood pressure (MAP) was 77 mmHg (range 73-80 mmHg).

Blood pressure measurements were done in 13 patients at follow-up, SBP mean was 112 mmHg (range 97-128 mmHg); DBP was 49 mmHg (range 30-75 mmHg); and MAP was 70 mmHg (range 58-90 mmHg).

There were no significant associations for initial and final SBP, DBP and MAP (p=0.7, p=0.2 and p=0.3 respectively; Wilcoxon test), however the number of patients was very small (n=2).

6.5.3. Haemoglobin

Initial and final haemoglobin (Hb) data were obtained from 13 patients. Initial mean Hb level was 8.9 g/dL (6.5-10.6 g/dL), and follow-up mean Hb level was 9.1 g/dL (range 6.1-10.9 g/dL). There was a trend for a higher Hb level at follow-up (p=0.07; Wilcoxon test).

6.5.4. Effect of Long-Term Blood Transfusion on Magnetic Resonance Imaging

Initial structural T2-weighted MRI scans of the patients were normal in 4 of the 17; there were unilateral (in one or several regions of a cerebral hemisphere) infarcts in 3 patients; and cerebral infarcts in both hemispheres (bilateral) in 10 patients.

At the end of the follow- up, 11 patients (65%) had unchanged and 6 (35%) had a worsening of their MRI studies. Table 6.3 and figure 6.4 relate changes of MRI over time and the different therapy modalities in these patients.

From table 6.3 and figure 6.4, two thirds of the patients on blood transfusion had worse MRI; another third had stopped blood transfusion. Whereas for those patients with

unchanged MRI, half were on blood transfusion, a quarter of the patients had stopped blood transfusion and the remainder were on alternative therapies.

MRI Changes over Time (n=Pats)	BTx	BTx Stopped	BTx Stopped HU	BTx Stopped BMT	BTx Stopped RV+HU
Unchanged	5	3	1	1	1
Worse	4	2			
Total	**9**	**5**	**1**	**1**	**1**

Table 6.3. Changes on MRI over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

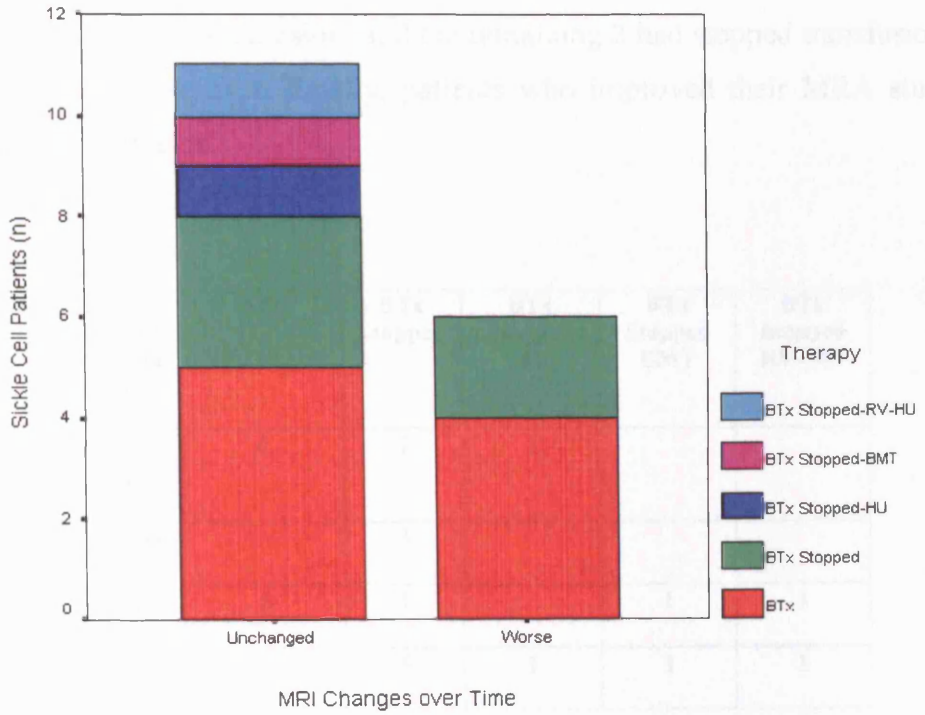


Figure 6.4. Changes on MRI over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

6.5.5. Effect of Long-Term Blood Transfusion on Magnetic Resonance Angiography

The sickle cell patients had the following grades of MRA abnormality initially, considering the worse grade of turbulence in each patient: normal MRA in 3 out of 17 patients; mild turbulence in 1; moderate turbulence in 5; severe turbulence in 4; artery occlusion in 2; and artery occlusion and collaterals in 2.

At the end of the follow- up, 6 patients (35%) had unchanged, 3 (8%) had improved, and 8 (47%) had worse MRA studies. Table 6.4 and figure 6.5 relate MRA changes over time and the different treatments in these patients.

From table 6.4 and figure 6.5, two third of the patients with worse MRA were on blood transfusion, whereas the remainder were on alternative therapies for SCD and on patient had had stopped transfusion. Of the patients who had unchanged MRA at follow-up, two third were on blood transfusion, and the remaining 2 had stopped transfusion but 1 / 2 continued on Hydroxyurea. Finally, patients who improved their MRA studies had stopped blood transfusion.

MRA Changes over Time (n=Pats)	BTx	BTx Stopped	BTx Stopped HU	BTx Stopped BMT	BTx Stopped RV+HU
Unchan-ged	4	1	1		
Improved		3			
Worse	5	1		1	1
Total	**9**	**5**	**1**	**1**	**1**

Table 6.4. Changes on MRA over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

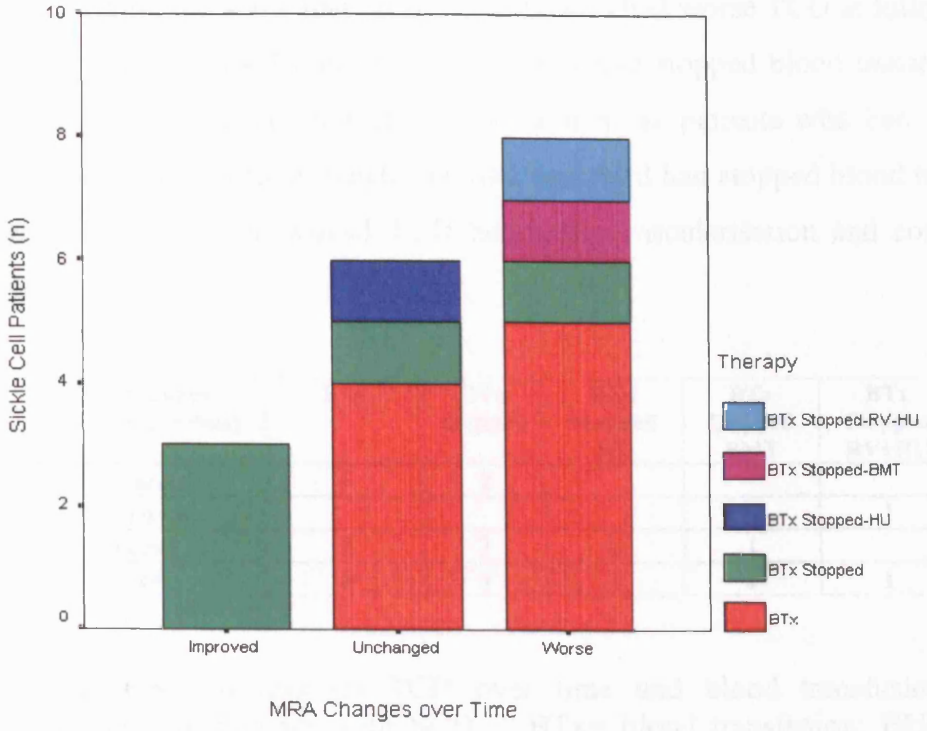


Figure 6.5. Changes on MRA over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

6.5.6. Effect of Long-Term Blood Transfusion on Trancranial Doppler Ultrasound

Initial transcranial Doppler studies (TCD; table 6.4) demonstrated that the patients had the following categories (taking into account the worst category following Adams' criteria [Adams et al 1992 and 1998]): normal in 3 of the 13 patients; mean maximum MCA (maxMCA) velocities $\geq$ 200 cm/sec in 1; mean maxMCA velocities < 70 cm/sec and ipsilateral lowest : highest mean maxMCA ratio <0.5 in 7; and undetectable MCA in 2 patients. There were no initial TCD data in 4 patients.

In the final TCD study, 6 patients (46%) had unchanged, 1 (8%) had improved, and 6 (46%) had worse TCD. Table 6.5 and figure 6.6 show changes over time of TCD and the different therapies in these patients.

Table 6.5 and figure 6.6 show that those patients who had worse TCD at follow-up, one third of them were on blood transfusion, one third had stopped blood transfusion, and the remainder were on alternative treatments. For those patients who had unchanged TCD, two third were on blood transfusion and one third had stopped blood transfusion. The patient who had an improved TCD had had revascularisation and continued on Hydroxyurea.

TCD changes over Time (n=Pats)	BTx	BTx Stopped	BTx Stopped HU	BTx Stopped BMT	BTx Stopped RV+HU
Unchanged	4	2			
Improved					1
Worse	2	2	1	1	
Total	**6**	**4**	**1**	**1**	**1**

Table 6.5. Changes on TCD over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

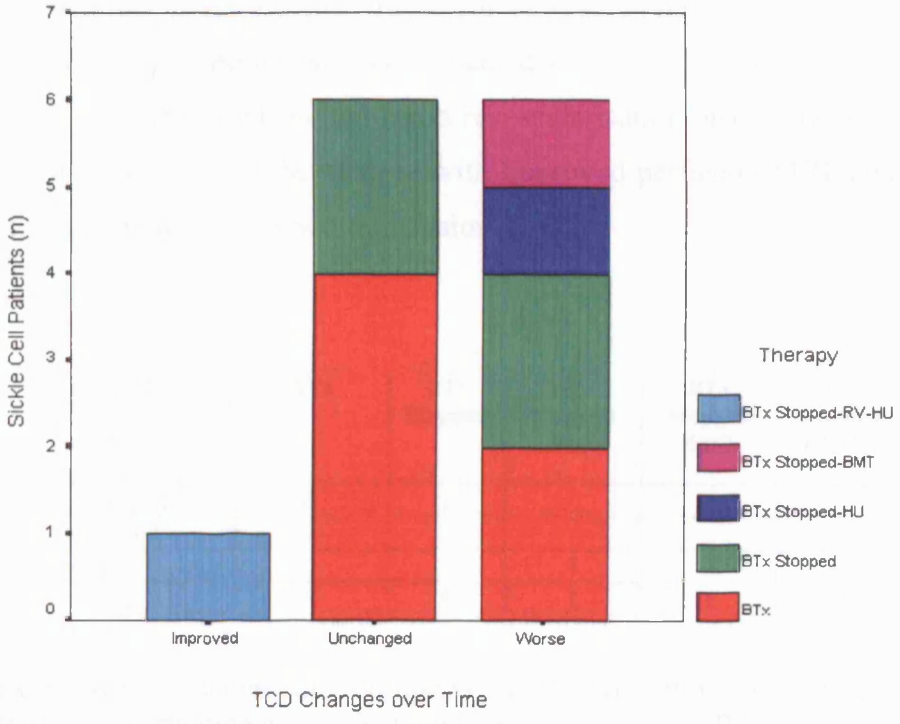


Figure 6.6. Changes on TCD over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

6.5.7. Effect of Long-Term Blood Transfusion on Cerebral Perfusion

Perfusion MRI studies at onset (Appendix-Table 3 [patients 1-16 and 20] and Appendix-Table 7) showed that three of the 17 patients with SCD had normal perfusion, whereas 6 had perfusion abnormalities confined to a region or regions of one cerebral hemisphere in the ACA, MCA, and /or PCA territories; and 8 patients had perfusion abnormalities in a region or regions of both hemispheres.

Follow-up perfusion MRI demonstrated that 3 patients (8%) had unchanged, 6 (35%) had improved, and 8 (47%) had a worse perfusion. Table 6.6 and figure 6.7 summarise the changes over time of perfusion MRI and the treatments of these patients.

From table 6.6 and figure 6.7, two thirds of the patients with worse perfusion were on blood transfusion therapy, whereas the remainder had stopped transfusion or had alternative treatments (bone marrow transplant or Hydroxyurea). Of three sickle cell patients with unchanged perfusion, one remained on blood transfusion and two had stopped transfusion, one of whom had had a revascularisation procedure and continued with Hydroxyurea. Finally, of the patients with improved perfusion MRI studies, more than half of them remained on blood transfusion.

Perfusion MRI Changes over Time (n=Pats)	BTx	BTx Stopped	BTx Stopped HU	BTx Stopped BMT	BTx Stopped RV+HU
Unchanged	1	2			
Improved	3	2			1
Worse	5	1	1	1	
Total	**9**	**5**	**1**	**1**	**1**

Table 6.6. Changes on Perfusion MRI over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

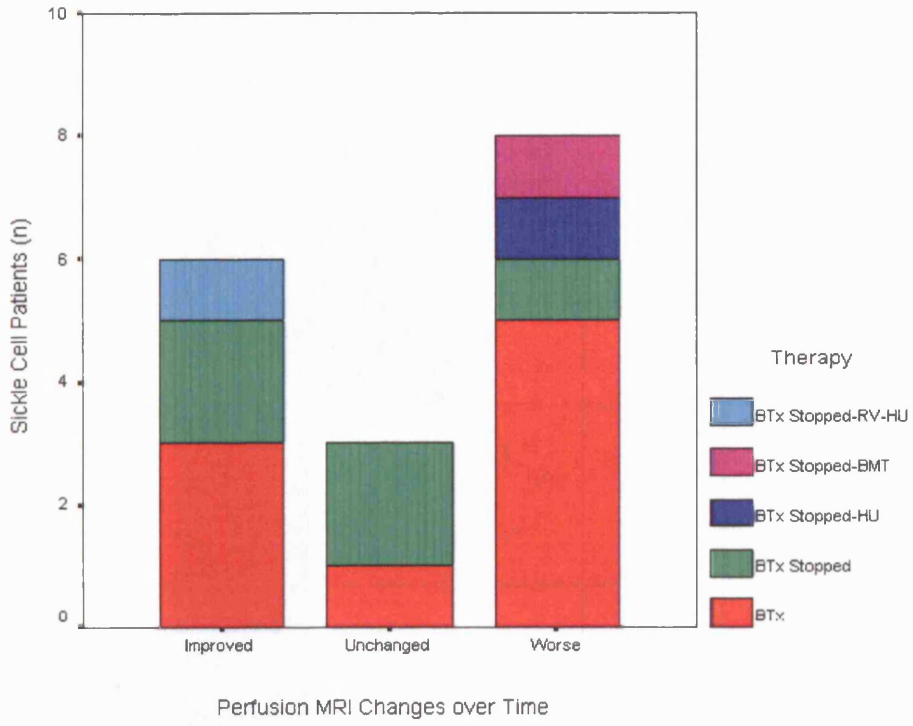


Figure 6.7. Changes on Perfusion MRI over time and blood transfusion treatments in patients with SCD. BTx= blood transfusion; HU= Hydroxyurea; BMT= bone marrow transplant; RV= surgical revascularisation procedure.

6.5.8. Comparison between Cerebral Perfusion and Conventional Neuroimaging and TCD in Long-Term Blood Transfusion

Worsening of the cerebral perfusion abnormality on perfusion MRI in 8 sickle cell patients who were on chronic blood transfusion was associated with worsening of MRI in 6, worsening of MRA in 8, and a worse TCD in 6 sickle cell patients (table 6.7). However, of 6 patients who improved their perfusion abnormality, only 3 had an improved MRA; and 1 patient had a better TCD. Only 3 patients had unchanged perfusion and 6 patients had unchanged MRA, whereas 11 patients had unchanged MRI and 6 unchanged TCD at follow-up (figures 6.8 and 6.9).

Change Over Time (n=Sickle Cell Patients)

Investigations	Better		Unchanged		Worse	
	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline	Normal Baseline	Abnormal Baseline
MRI (n=17)			4 [2§ 1§HU]	7 [1§ 1§HU+RV 1§BMT]	1 [1§]	5 [1§]
MRA (n=17)		3 [3§]	3 [1§ 1§HU]	3		8 [1§ 1§BMT 1§HU+RV]
TCD (n=13)		1 [1§HU+RV]	-	6 [2§]	2 [1§HU]	4 [2§ 1§BMT]
Perfusion MRI (n=17)		6 [2§ 1§HU+RV]	1 [1§]	2 [1§]	1 [1§HU]	7 [1§ 1§BMT]

Table 6.7. Changes over time of MRI, MRA, transcranial Doppler ultrasound and perfusion MRI in relation to recurrent neurological symptoms in sickle cell patients on long term blood transfusion therapy.

BTx: Blood transfusion therapy; TIA: Transient ischaemic attack.

HU : Number of sickle cell patients on Hydroxyurea therapy;

§ Number of patients who stopped blood transfusion therapy during follow-up

§BMT Number of patients who stopped blood transfusion therapy and had bone marrow transplant during follow-up

§HU+RV Number of patients who stopped blood transfusion therapy, had surgical revascularisation procedure and continued with Hydroxyurea during follow-up

No data because no improvement can be demonstrated from a normal study or MRI

6.5.9. Alternative Therapies in SCD and their Effect on Magnetic Resonance Studies and TCD

6.5.9.1. Blood Transfusion Discontinued Followed by Hydroxyurea

A male patient with sickle cell anaemia (table 6.1, No. 15) presented with posterior territory TIAs at 15 years of age, his initial investigations (MRI, MRA, perfusion MRI and TCD) were all normal and he was initially on blood transfusion, which was discontinued as he was asymptomatic and he started on Hydroxyurea. He had recurrent headaches. Four years later, follow-up investigations showed abnormal perfusion in the right temporal areas (increased MTT and decreased CBF), and abnormal TCD with decreased velocities < 70 cm/sec in the right middle cerebral artery, but he had unchanged MRI and MRA.

6.5.9.2. Blood Transfusion Discontinued Followed by Revascularisation Procedure and Hydroxyurea

This patient, a 12-year-old boy with sickle cell anaemia (Table 6.1, pat No. 14), presented with anterior territory TIAs (transient right hemiparesis), headaches and learning difficulties. His initial investigations showed multiple small watershed infarcts bilaterally on MRI, moderate turbulence in the anterior circulation (MCAs/ACAs), bilateral perfusion abnormalities (increased MTT and decreased CBF) in watershed regions (ACA/MCA/PCA territories), and abnormal TCD (right MCA). The patient was initially on blood transfusion, however, because of his recurrent severe symptoms, he underwent a left indirect frontal revascularisation procedure, stopped blood transfusion and continued on Hydroxyurea. Afterwards, he had recurrent headaches and ongoing learning difficulties but not TIAs. After 3 years, follow-up investigations showed an improvement (mainly) in his cerebral perfusion in some areas bilaterally, although there were two regions on the left hemisphere with impaired perfusion, but his MRI was unchanged. Follow-up MRA showed improvement in the turbulence of the left M1 segment (the same side of the revascularisation procedure), however his cerebrovascular disease was worse on the right. However, the patient improved his cerebral perfusion and his symptoms.

6.5.9.3. Blood Transfusion Discontinued – Bone Marrow Transplant

This 10-year-old girl with sickle cell anaemia, presented with stroke (right hemiparesis and aphasia). Her initial investigations showed a left frontal parietal infarct and on the right, deep watershed infarcts. She had cerebrovascular disease with severe turbulence (internal carotid artery, MCA) and collaterals bilaterally, abnormal TCD with low velocities (<70 cm/sec) bilaterally and abnormal bilateral perfusion in the MCA territory (increased MTT and decrease in CBF). She started blood transfusion but continued to have recurrent anterior territory TIAs. Two years later, blood transfusion was stopped and she received a bone marrow transplant and her symptoms improved. In the follow-up investigations, after 3 years from the initial studies, she had a worse perfusion MRI with more extended perfusion abnormality bilaterally in the MCA territories, however her MRI was unchanged. There was progression of the cerebrovascular disease with more collaterals bilaterally, and she had undetectable MCA in both sides on TCD.

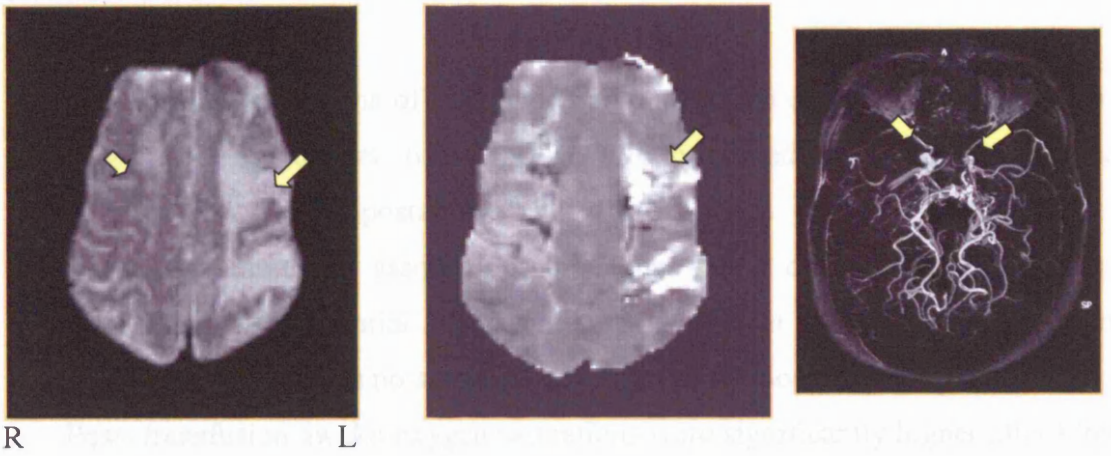


Figure 6.8. Long-Term blood transfusion. 21-year old female, severe SCD (HbSS), bilateral MCA stroke, leg ulcers,on chronic blood transfusion. ***T2-w MRI*** . Middle cerebral artery (MCA) infarction bilaterally (left >right). Abnormal ***Perfusion MRI.*** Increased mean transit time of the passage of IV contrast bolus in the MCA territories bilaterally more on the left than the right. ***MRA***. Occlusion of the right MCA & anterior cerebral artery (ACA), severe turbulence in the left MCA and moyamoya collaterals. R: right; L: left.

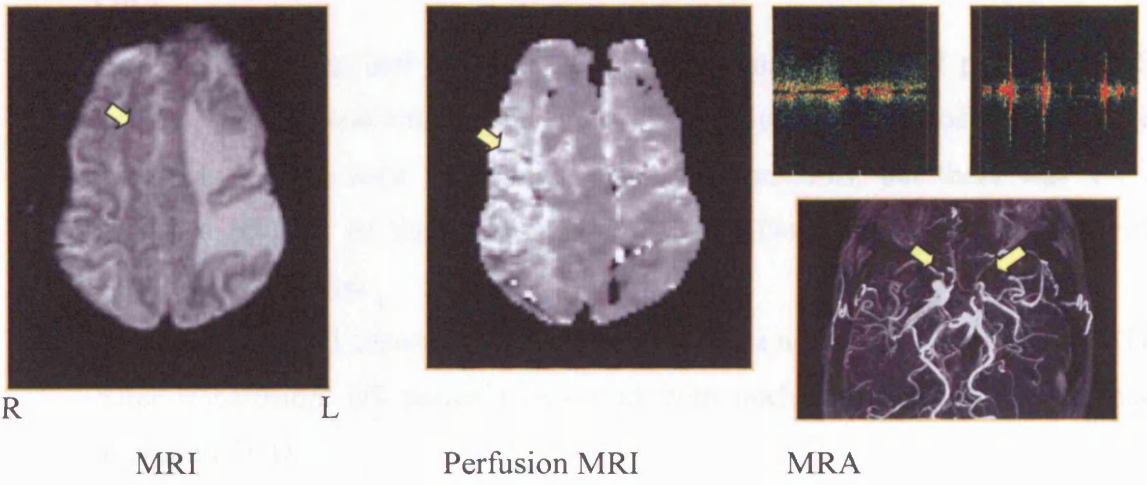


Figure 6.9. Long-term blood transfusion, after 2 years (the patient stopped blood transfusion a year before due to erythrocyte antibody formation). ***T2-w MRI***. Bilateral infarctions, patchy white matter ischaemic lesions, bilateral basal ganglia infarcts & generalised atrophy. Impaired ***Perfusion MRI***. Increased mean transit time of the passage of IV contrast bolus in the MCA territories bilaterally (cortical and subcortical regions) with impaired cerebral perfusion on the cerebral perfusion on the right MCA region. : ***MRA.*** Both proximal MCAs and left terminal ICA are narrowed, moyamoya collaterals. Transcranial Doppler ultrasound ***(TCD):*** decreased MCA blood flow velocities bilaterally.

6.6. Summary of Results

6.6.1. Short-Term Blood Transfusion

- Neurological symptoms of the sickle cell patients on chronic blood transfusion (n=8) were as follows: 6 had stroke (1/6 presented with coma), 1 anterior territory TIA, and one posterior territory TIA.
- There were trends for associations for higher values of systolic blood pressure (SBP) and mean arterial blood pressure (MAP) in the patients after blood transfusion. There was no association for diastolic blood pressure (DBP).
- Post- transfusion awake-oxygen saturations were significantly higher after blood transfusion.
- The mean haemoglobin level was 10 g/dL (from the nearest blood test to the investigations).
- All the patients had cerebral infarction on their MRI, which remained unchanged after blood transfusion.
- Seven out of 8 patients had abnormal and one patient had normal MRA. After blood transfusion, 4/8 patients had unchanged, 3/8 had worse and 1/8 improved MRA.
- 7 of the 8 patients had abnormal and one patient had normal perfusion MRI initially. After blood transfusion, there was an improvement of the perfusion abnormality in at least a cerebral region in 5 patients, but there was not a complete reversal of the abnormal perfusion. Three patients remained with unchanged perfusion.
- Seven patients had abnormal, and one patient had a normal pre-transfusion TCD. After transfusion, 6/8 patients remained with unchanged, and 2 patients had improved TCD.

6.6.2. Long-Term Blood Transfusion

- The neurological symptoms at presentation in the 17 patients of this cohort were as follows: 2 had coma with stroke, 1 coma with posterior territory TIA, 7 stroke, 4 anterior territory TIAs, 2 posterior territory TIAs, and 1 headaches.

- The recurrent neurological symptoms were as follows: 1 patient had coma (with posterior leukoencephalopathy on MRI), 1 had stroke, 6 headaches, 1 seizures, 6 anterior and 1 posterior territory TIA, and one patient did not have further symptoms.
- Seventeen patients were initially on blood transfusion; during follow-up, 9 patients remained on blood transfusion, 5 stopped blood transfusion, 3 patients stopped blood transfusion but continued on Hydroxyurea (n=1), had revascularisation and continued on Hydroxyurea (n=1), and one patient underwent bone marrow transplantation.
- There were no significant differences between the initial and follow-up SBP, DBP and MAP.
- There was a trend for higher haemoglobin levels at follow-up (initial mean Hb 8.9 [6.5-10.6] g/d/L; final mean Hb 9.1 [6.1- 10.9] g/dL).
- Initial MRI was normal in 4/17 patients, and abnormal in 13 patients. At follow-up 11 patients had unchanged, and 6 patients had worse MRI.
- Initial MRA was normal in 3 and abnormal in 14 patients. In the final study, 6 patients had unchanged, 3 improved and 8 patients had worse MRA.
- Initial TCD was normal in 3/13 patients and abnormal in 10. At follow-up 6 patients had unchanged, 1 improved, and 6 patients had worse TCD.
- Perfusion MRI studies were initially normal in 3/17 patients and abnormal in 14 patients. Final perfusion MRI showed unchanged perfusion in 3 patients, improved in 6, and 8 patients had worse perfusion.

6.7. Discussion

This study showed two different effects of the blood transfusion therapy on cerebral perfusion in relation to the timing. Whereas in the short- term there was an improvement of the cerebral perfusion (in at least one cerebral region) shortly after blood transfusion in more than half of the patients, in the long-term, perfusion abnormality and MRA was worse or unchanged in two third of the sickle cell patients on chronic transfusion, with a worsening of the structural MRI in a further third of these patients (35%). However, there was a very low prevalence of recurrence of severe neurological complications such as stroke in only one patient (6%) and coma in another

one (6%) in the long-term study, showing the beneficial effect of blood transfusion in reducing the risk of recurrent stroke (but not infarction) in this population, but there was no reversal of the perfusion abnormality and the cerebrovascular disease.

In this study, there was an improvement of the regional abnormal perfusion in 5/8 of the sickle cell patients shortly after blood transfusion but not a complete reversal of the perfusion abnormality. The regional improvement on perfusion MRI maps was seen in the decrease in the mean transit time (MTT) or in an increase in cerebral blood flow (CBF) and/or cerebral blood volume (CBV) in very localised areas of the brain. This improvement of the cerebral blow flow was reported previously (Prohovnik et al 1989, Hurlet- Jensen et al 1994, Kirkham et al 2001) demonstrating the immediate beneficial effects of the cerebral vasculature of the blood transfusion by improving the rheology of the red cells in this inherited anaemia (Ballas et al 2002) and the cerebral perfusion. In addition, blood pressure and oxygen saturations were higher after transfusion, which might contribute to the improvement of the cerebral blood flow and tissue oxygenation.

On the other hand, MRA and TCD were only improved in a small proportion of patients, and there were no changes on MRI, demonstrating that perfusion was the most sensitive modality of investigation to monitor changes which may be useful to guide management in individual patients (Kirkham et al 2001). Blood transfusion might affect blood vessels, especially in those patients with underlying cerebrovascular disease by increasing the turbulence in some arterial segments as was shown in this study, however these patients did not have a worsening in their perfusion abnormality. On the other hand, there was extensive cerebrovascular disease in a proportion of these patients and some of those appeared to have improvement or worsening of the turbulence in different vessels on the same MRA; therefore pre- and post- transfusion MRA may be misleading in terms of the real effect of blood transfusion on cerebral perfusion in these patients.

Long-term blood transfusion was beneficial in reducing stroke recurrence, and blood transfusion is the mainstay of treatment for prevention of recurrent stroke (Ohene-Frempong et al 1991, Serjeant 1997, Ballas et al 2002, Kirkham and deBaun 2004), but blood transfusion did not stop the progression of cerebrovascular disease and the perfusion abnormality in these patients in an important proportion of these patients

(47%). However, the only patient who had recurrent stroke had had stopped the transfusion therapy due to erythrocyte antibody formation. In addition, 16 of the 17 patients continued having recurrent neurological symptoms, probably as an expression of the perfusion abnormality and cerebrovascular disease.

Only nine of the 17 sickle cell patients in this study continued on chronic blood transfusion, and the remainder stopped blood transfusion or continued with alternative therapies. Nevertheless, more than half of the patients on blood transfusion (5/9 patients) still had a worsening of their cerebral perfusion and cerebrovascular disease. Although a previous study showed an improvement of the cerebrovascular disease after chronic transfusion (Russell et al 1984), this study showed that there is progression of cerebrovascular disease and blood transfusion could have a damaging effect on the cerebral blood vessels secondary to the chronic iron overload, in addition to infectious/inflammatory mechanisms (Belcher et al 2000) secondary to the frequent infections and sickle crisis that these patients usually suffer from (Russell et al 1984).

Iron overload has been associated to induced vascular dysfunction by accelerating thrombus formation after arterial injury, by increasing vascular oxidative stress and by impairing vasoreactivity in animal models (Day et al 2003). In addition, iron injury in blood vessels has been associated with carotid atherosclerosis (Schmitz 2003), as iron might be toxic to the endothelium by participating in the generation of powerful oxidant species such as hydroxyl radicals and in lipid peroxidation (Shah and Alam 2003). Iron has found to cause vasocontriction in animal models (Kuang et al 2003). However the susceptibility of an individual for iron-related endothelial injury might be more important than the overall iron body status (Shah and Alam 2003), as this study showed a proportion of patients on blood tranfusion had unchanged or improved MRA at follow-up. In addition there was an increase in the haemoglobin levels of these patients at follow-up, which might contribute to the increase of free iron molecules secondary to red cell destruction and possible iron-related endothelial damage. The relationship between increased haemoglobin level and cerebrovascular disease in SCD could be explored in further studies.

Transcranial Doppler ultrasound was unchanged in 6 patients and worse in other 6 out of 13 patients at follow-up, similar proportions that were found for MRA,

demonstrating that TCD is an adequate technique to monitor cerebrovascular disease in patients on chronic transfusion or with alternative treatments in the long term.

Patients who stopped blood transfusion had a variety of recurrent neurological symptoms, mainly headaches and TIAs, in addition to the patient who had recuurent stroke. In addition, these patients had unchanged or worse MRI in similar proportions, however those who stopped blood transfusion had improved MRA at follow-up, which might support the hypothesis of the iron endothelial injury (Shah and Alam 2003), or that these patients were less exposed to infections or SCD crisis during the follow-up or may reflect a selection bias. There were also smaller proportions of patients with unchanged or worse MRA, and two third of the patients had improved or unchanged perfusion MRI at follow-up. This finding demonstrates the individual variability of SCD in relation to the phenotypical expression of this condition (Weatherall et al 1995) and its treatment. However, most of the patients who stopped blood transfusion had milder baseline investigations and symptoms (severity and frequency) in relation to other patients, and blood transfusion therapy was discontinued (only in two patients had poor compliance or erythrocyte antibody formation).

The small number of patients who had alternative treatments did not have stroke recurrence or new infarction (covert) on MRI. The patient who had revascularisation and continued on Hydroxyurea had progression of the cerebrovascular disease (CVD), especially on the other side of the revascularisation, but he had improved cerebral perfusion and was asymptomatic, demonstrating that this therapy could be an alternative to those patients with poor compliance to blood transfusion, severe CVD and severe recurrent neurological symptoms (Vernet et al 1996, Ganesan et al 2001, Fryer et al 2004). On the other hand, the patient who had bone marrow transplant (BMT) had a worse MRA and cerebral perfusion but did not have recurrent stroke or cerebral infarction on MRI. This therapy is considered curative for SCD (Walters et al 2000), and, although there was a study showing reversal of the CVD on MRA in patients who had received a BMT (Steen et al 2001), the effectiveness of this treatment on improving cerebral perfusion in the long term is uncertain. Finally, one patient on Hydroxyurea, after discontinuing blood transfusion, had preserved structural neuroimaging but his cerebral perfusion (previously normal) and TCD were worse at follow-up. Although there is an ongoing trial on Hydroxyurea for prevention of secondary stroke as

alternative treatment of blood transfusion for children with SCD and neurological complications (Ware et al 2004), the protective effect of this drug to halt cerebrovascular disease and cerebral perfusion abnormality in patients with SCD in the long term requires further exploration.

6.8. Conclusion

This study showed that, in the short term, blood transfusion improved cerebral perfusion in more than half of the patients with sickle cell disease and neurological complications. However, in the long-term, although blood transfusion therapy was effective in preventing stroke recurrence, it was rarely associated with reversal of cerebrovascular disease and cerebral perfusion abnormality. The effect of the blood transfusion and iron overload on the cerebral vasculature together with infectious/inflammatory mechanisms might contribute to the progression of the CVD in these patients, however future research will be needed to study this association.

Alternative therapies in patients with SCD prevented stroke recurrence in this study; however bone marrow transplantation or Hydroxyurea did not halt the progression of cerebrovascular disease and perfusion abnormality in two patients. Surgical revascularisation in conjunction with Hydroxyurea improved cerebral perfusion in one patient, and this alternative treatment could benefit those sickle cell patients who have severe recurrent neurological symptoms and irreversible and severe cerebrovascular disease, particularly if they are poorly compliant with blood transfusion therapy.

Chapter 7: Seizures in Sickle Cell Disease: Perfusion Abnormality, Vasculopathy and Ischemia

7.1. Introduction and Aims

7.1.1. Introduction

Partial or generalized seizures affect 12-14% of patients with sickle cell disease (SCD), herald stroke in 10-33% (Liu et al 1994, Adams 1994) and are associated with silent infarction (Miller et al 1999). Triggers include central nervous system infections (Danesi et al 1983), chest syndrome (Henderson et al 2003), nephrotic syndrome (Coley et al 1999, Horton et al 1995), trauma (Danesi et al 1983), hypertension (Hamdan et al 1984), *Parvovirus* infection (Wierenga et al 2001), drugs used in parenteral pain relief (Liu et al 1994, Nadvi et al 1999, Zolezzi et al 2001), blood transfusion (Royal et al 1978, Siegel 1993) and bone marrow transplantation (Abboud et al 1996). However, the pathophysiology is poorly understood. Studies of cerebral blood flow with ^{133}Xenon inhalation have shown that encephalopathic patients with sickle cell disease and seizures have areas of regional hypoperfusion, some of which may resolve at follow-up (Huttenlocher et al 1984). More recently, emergency MR in patients with sickle cell disease and acute seizures has shown sinovenous thrombosis (van Mierlo et al 2003) or changes on fluid-attenuated inversion recovery (FLAIR) or diffusion MR compatible with acute breakdown of the blood-brain barrier (Henderson et al 2003, Coley et al 1999). There are few data in conscious patients with active acute or chronic epilepsy, however, and vascular imaging is rarely performed.

In a previous cross-sectional study using Gadolinium MRI to image perfusion in 48 SCD patients with central nervous system involvement (Kirkham et al 2001), of six with a history of seizures, two had brain infarction, both with associated perfusion abnormality, one of which was beyond the area of infarction. However, some of the patients had had remote seizures several years before.

7.1.2. Aims

The aim of this study was to examine the prevalence of cerebral vasculopathy in SCD patients with and without seizures and of MR perfusion abnormality in those with recent seizures.

7.2. Subjects

7.2.1. Seizure Patients

Forty-seven SCD patients were recruited from joint Haematology/Neurology clinics described in chapter 2. Forty-one patients with SCD were all neurologically symptomatic (with seizures [n=6]; and without seizures [n=35]), and an additional six patients, who had abnormal transcranial Doppler (TCD) ultrasound study and had suspected cerebrovascular disease were recruited. Of the 47 patients, 44 had sickle cell anaemia [HbSS], 2 had haemoglobin SC disease [HbSC], and one had sickle cell β^0 thalassaemia. The median age was 12 years (range 1.7-27 years, 23 males). Data on clinical presentation were obtained from the clinics or from the patients' medical records.

7.2.2. Controls

The control group of children with homozygous sickle cell anemia had no evidence of neurological disease (n=29; 15 male) over a prolonged follow-up period but had undergone unsedated routine MRI and MRA screening (but not perfusion) over the age of six (median age 9.9, range 6.6-18.2 years) from within a clinic-based but otherwise unselected prospective cohort recruited from joint Haematology/ Neurology Clinics recruited by Dr Fenella Kirkham, Reader in Paediatric Neurology, Institute of Child Health, as a part of an ongoing study of the risk factors for stroke and cerebrovascular disease (CVD) in SCD.

7.4. Methods

7.4.1. Conventional Neuroimaging, Perfusion MRI, Transcranial Doppler Ultrasound, Oxygen Saturation, Blood Pressure Measurements and Haematological Parameters

The methods were described in chapter 3. The patients with neurological symptoms or abnormal TCD (n=47; seizure, non-seizure and abnormal TCD) underwent magnetic resonance (MRI, DWI, MRA and perfusion MRI); transcranial Doppler ultrasound investigations; blood pressure measurements, and 3 minutes' awake-pulse oximetry [SpO_2]. Data on haematology (haemoglobin level) were obtained from the patients' medical records as close as possible to the scan.

The control group (n=29) had undergone unsedated routine MRI and MRA screening over the age of seven and transcranial Doppler ultrasound but these group did not have pulse oximetry or perfusion MRI.

For this study the TCD findings were considered abnormal if one or more of the following findings were present: 1) an MCA ratio (lowest: highest) velocity < 0.5; 2) an ACA: MCA mean velocity ratio greater than 1.2; 3) mean MCA velocity greater than 170 cm/sec and less than 200 cm/sec, deemed conditional by Adams and colleagues (Adams et al 1997); 4) a mean MCA velocity equal or greater than 200 cm/sec deemed critical and premonitory for stroke by Adams and colleagues (Adams et al 1997); 5) a mean MCA velocity less than 50 cm/sec (Adams et al 1997, Kirkham et al 2001a, Zafeiriou et al 2004).

7.4.2. Neurophysiology

An electroencephalogram (EEG) was recorded in patients who had seizures, using standard digital EEG equipment (Nicolet 'Bravo'), and electrode placements. EEGs were reported using conventional clinical methods of visual inspection.

7.4.3. Statistical Analysis

The data were analysed using SPSS for Windows version 10.0. One-way ANOVA was used to examine the association of continuous (parametric) and categorical data, and the Kruskal Wallis test to examine associations of non-parametric continuous data and categorical data between seizure, non-seizure and control groups. Binary logistic regression was used to examine the association of continuous and categorical data in relation to a binary outcome (seizure versus non-seizure group). Chi square test and Cramer's V statistics were used as measures of strength of the relationship between categorical variables and groups of patients (seizure and non-seizure group [neurologically symptomatic but without seizures]) and controls. Level of significance was set at $p< 0.05$ and a trend for significance was defined as $p \geq 0.05$ and $p \leq 0.1$.

SCD patients were placed into one of three groups for the purposes of analysis: 1. seizure, 2. non-seizure (neurologically symptomatic but without seizures), and 3. control (neurologically asymptomatic).

7.5. Results

7.5.1. Patients: Neurological Symptoms and Seizure Type

The neurological problems in this group of sickle cell patients (n=47) included: stroke (n=9), transient ischemic attack (TIA; n=8); seizures (n=6), headaches (n=9), and behavioural and/or learning difficulties (n=9); six patients had abnormal transcranial Doppler (TCD) ultrasound study and had suspected cerebrovascular disease. The controls (29 SCD patients) did not have neurological signs or symptoms), but one was known to have an abnormal TCD.

Of the 6/47 (13%) neurologically symptomatic patients with seizures, five had HbSS, and one had sickle cell β^0 thalassaemia. One also had overt cerebral infarction (stroke) and another also had recurrent TIAs. The median age of the seizure patients was 10.1 years (range 1.7-20.3 years); two were male. Two had clinically generalized seizures, one with staring spells and the other with a non-febrile generalized tonic-clonic seizure.

The other four patients had seizures suggestive of focal onset. One had a hemiparesis and focal facial twitching at presentation; during follow-up she developed staring spells. A patient who had cerebral infarction had hallucinations and visual phenomena; one with recurrent TIAs and headaches had tonic or jerking seizures of the right arm and leg; and one had staring spells and nocturnal seizures with right sided twitching.

7.5.2. Neurophysiology

An electroencephalogram (EEG) was recorded in all seizure patients at a median of 8.5 days (range 4-210 days) after the last seizure and a median of 4 (range 0-56) days after the neuroimaging in five patients, and 64 days before the neuroimaging in one patient. EEG was initially abnormal in 4/6 patients (table 7.2), showing (a) left temporal focal slow activity and isolated sharp waves (patient 5); (b) an excess of left focal slow activity with further slowing following over-breathing (patient 1); (c) minor focal abnormalities over the right central region (patient 3); and (d) interictal runs of focal spikes over the right Sylvian area (patient 4). Two patients had a normal EEG. At follow up after one year in two patients, one (patient 1), who had had an EEG showing focal slow activity exacerbated by over-breathing, developed sharp and spike wave activity over the same region; the other patient (patient 2), who had had a previously normal EEG, developed bilateral spike activity.

7.5.3. Transcranial Doppler Ultrasound

Mean MCA velocity was significantly different between the children with seizures, those with other neurological symptoms and the asymptomatic controls (table 7.1). Post-hoc analysis of the three groups showed a trend for increased velocities in the seizure group and controls in relation to the non-seizure group ($p=0.1$ and $p=0.06$, respectively) but no difference between the seizure and the control groups ($p=0.7$).

Following Adams' criteria, seizure patients had more abnormal TCD studies than the non-seizure and control groups (table 7.1). Two patients with seizures had TCD mean MCA velocities higher than 200 cm/sec, defined as critical (Adams et al 1997), both ipsilateral to EEG focal findings, whereas no non-seizure patient in this sample did. Two seizure patients had decreased mean MCA velocities less than 50 cm/sec

unilaterally and one patient had turbulent signal in one MCA but blood flow velocities within the normal range. In the seizure group there were more TCD studies which were at least conditional (mean MCA maximum velocity equal or more than 170 cm/sec) than in the non-seizure patients and controls (table 7.1). There was no difference between seizure, non-seizure and control groups for the proportion with mean maximum MCA velocity less than 50 cm/sec, which is associated with arterial occlusion or severe stenosis ($p= 0.5$, χ^2 test). During follow-up of those with seizures and a MCA velocity more than 200 cm/sec, one continued to have a MCA velocity over 200 cm/sec, and the other progressed to a pattern of artery occlusion or severe artery stenosis, with decreased mean MCA velocities (<50 cm/sec) in the affected cerebral blood vessel.

7.5.4. MRI, MRA and Diffusion Weighted Imaging

In the seizure group, three patients had normal MRI, one had silent infarction, one had an overt infarct and old deep white matter silent infarcts, and one patient had cerebral atrophy. Of the 41 non-seizure neurologically symptomatic patients, T2-weighted MRI was normal in 24 patients, 8 had overt infarction, 8 had silent infarction in 8 and one had cerebral atrophy. Of the neurologically asymptomatic group, 10 of 29 patients had silent infarction.

Turbulence on MRA was found in 4/6 seizure patients, in 19/40 non-seizure patients, and 20/29 controls. There were no abnormal DWI studies in any patient. Abnormal MRI and MRA were not more common in those with seizures compared to the non-seizure and control group (table 7.1). Silent infarct on MRI was not more common in the seizure group compared to those without seizures and controls (table 7.1).

7.5.5. Perfusion MRI (Dynamic Susceptibility Contrast –MRI [DSC-MRI]

Perfusion MRI (DSC-MRI), reported only for the seizure group, was performed at a median of 30 days (range 0-365 days) from the last seizure. Two of six with seizures were on a monthly blood transfusion programme and perfusion MRI was done at a mean of 29 days after the last transfusion (one day prior to transfusion in both patients).

All six seizure patients had abnormal perfusion (figures 7.1 and 7.2), characterised by cerebral regions of increased mean transit time (MTT) and decreased cerebral blood flow (CBF) in five, or an increase in time to peak (TTP) in one. In five patients, perfusion abnormalities corresponded to the side of the abnormal EEG, and one patient had a region of abnormal perfusion in the right temporal region with a normal EEG (table 7.2).

7.5.6. Blood Pressure Measurements, Haematological Parameters and Pulse Oximetry

Median systolic, diastolic and MAP blood pressures in the seizure group were slightly higher in comparison with the non-seizure group (table 7.1). There was a trend for seizure patients to have higher mean haemoglobin levels than the non-seizures patients and controls (table 7.1). There was a trend for a higher SpO_2 in the seizure compared with the non-seizure group (table 7.1).

7.5.7. Management

The treament of seizures in our patients with SCD and vasculopathy proved difficult. Five of the six patients had seizure recurrence despite either anticonvulsant therapy (three patients) or blood transfusion (one patient) or both (one patient). Only one child, who had a single generalized seizure, had no treatment; she had had no recurrence at the time of follow-up.

7.6. Discussion

It is well-recognised that seizures are more common in SCD than in the general population (Adams et al 1994, Prengler et al 2002). For example, in a Nigerian population, 10% had seizures and epilepsy was associated with a higher mortality (Adamolekum et al 1993). Liu and colleagues found 21/152 SCD (14%) patients had seizures; more than 50% were generalized (Liu et al 1994). Forty-five percent of seizure patients had abnormal neuroimaging and almost half had an abnormal EEG. Seizures are a strong predictor of stroke in elderly adults in the general population (Cleary et al

2004). However, although sickle cell disease is the commonest cause of stroke in children (Earley et al 1998), any association between seizures and intracranial vasculopathy or focal ischaemia in this condition has received relatively little attention.

In this study, this study demonstrated that the patients with recent SCD associated seizures had abnormal perfusion MRI ipsilateral to any EEG abnormality. Three patients had abnormal regional perfusion with decreased CBF and increased MTT despite normal T2-weighted MRI, two had abnormal regional perfusion within the same territory as T2-weighted MRI abnormalities and one patient had decreased perfusion in association with generalised cerebral atrophy. Perfusion abnormalities involved cortical and subcortical areas and probably represent areas of relative ischemia which may be the anatomical source of seizure discharges. TCD studies showed both higher mean velocities in the seizure group and more abnormal individual TCD studies (5/6 in those with seizures who also had perfusion abnormalities on MRI). In 3, abnormal TCD (2 had critical velocities > 200) coincided with abnormal ipsilateral abnormal MRA. Vascular insufficiency appears to be more common in those with compared with those without seizures based on the TCD group comparisons.

Seizures may be the presenting symptom of overt stroke or relative vascular insufficiency. In a study of very young SCD children, seizures were documented in 3/39 patients between 7 to 48 months of age; all had cerebral infarction (Wang et al 1998). In the Cooperative Study of Sickle Cell Disease, seizures were an independent risk factor for silent infarction (Miller et al 1999). However, in our previous series of children undergoing MR, only 2/6 of those with seizures had T2-weighted abnormality and of the current prospective cohort, 3/6 had normal scans. Seizures may also be associated with large vessel disease, although our data from this and the previous series (Kirkham et al 2001) suggest that MRA be normal even when TCD is abnormal. Recent data from the STOP study suggests that TCD may pick up large vessel abnormality of haemodynamic importance before MRA (Abboud et al 2004) and our data would be in line with that. It is possible that some of these TCD abnormalities, particularly those associated with normal MRA, represent reversible vasospasm in response to an environmental trigger, rather than fixed stenosis.

The pathophysiology of SCD associated seizures has not previously been studied in detail. Neurologically asymptomatic SCD patients have global cerebral hyperemia secondary to anaemia (Prohovnik et al 1989) and may fail to increase CBF with hypercapnia (Prohovnik et al 1989) suggesting that the increased cerebral blood flow is secondary to adaptive vasodilatation and the vascular reserve capacity may be reduced. However, Huttenlocher and colleagues found significantly reduced mean CBF in children with stupor and seizures, with additional regional reductions despite normal cerebral angiography (Huttenlocher et al 1984) At follow-up, mean CBF was within normal limits, but some of the regional abnormalities persisted, particularly in the parietal regions. In a previous MR perfusion study, two patients with seizures had perfusion abnormalities despite normal MRA (Kirkham et al 2001). In this study of children with active seizures, although two children had normal MRA, the majority of patients had MRA evidence of vasculopathy and all had abnormal MR perfusion ipsilateral to any EEG abnormality. Seizures in young children often occur during situations which increase metabolic demands, particularly infections or stress. Our data suggest that the vascular insufficiency may play a direct role.

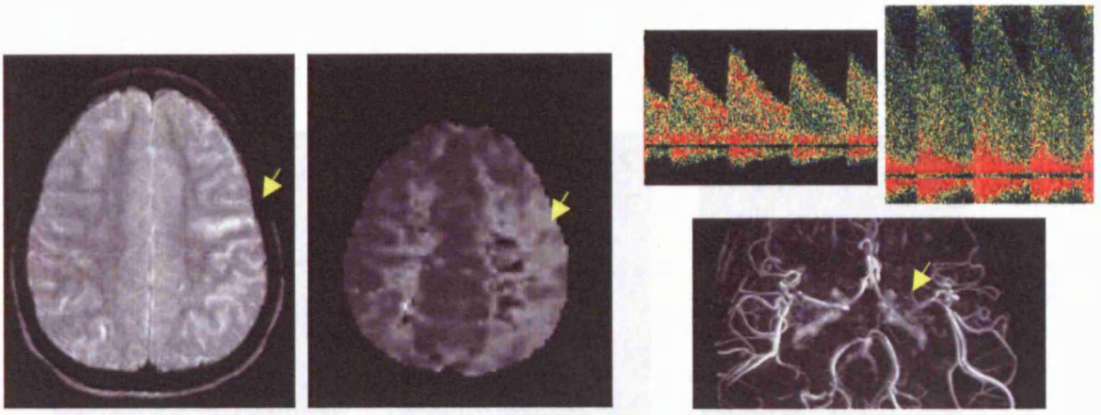
One SCD patient with seizures had a perfusion study performed ictally which showed decreased perfusion ipsilateral to a focal seizure and ipsilateral to the hemisphere with silent infarction in the deep white matter. It is possible that CBF is unable to increase focally to meet increased metabolic demand in a maximally vasodilated cerebral circulation, as has been shown in Sturge-Weber syndrome, for example (Aylett et al 1999). An alternative explanation is that the deep white matter containing the areas of silent infarction is deafferented. In an animal model, surgical cortical deafferentation in a hemisphere led to an increase of asynchronic electrical activity, with generation of seizures in intact regions and delayed paroxysmal activity in the deafferented hemisphere. This study showed that deafferented neurons needed more time to reach a firing threshold as the time lag in the propagation of electrical activity between the intact and undercut areas was increased (Topolnik et al 2003). We speculate that chronic ischaemia in the deep white matter could produce chronic axonal damage similar to a 'cortical deafferentation', where, in some cases, the seizure activity could be generated in the normal hemisphere, and not in the hemisphere with abnormal perfusion and risk of white matter ischaemia.

Treatment of seizures in SCD is not evidence-based. The presence of cerebrovascular disease and anaemia in this population may be a reason to consider alternative therapies beside antiepileptic drugs (AED). In animal models, some AEDs may have neuroprotective effects in brain ischaemia by decreasing excitatory transmission or enhancing neuronal inhibition either through receptors (e.g Felbamate) or ion channels (e.g carbamazepine, valproate, lamotrigine [Calabresi et al 2003]), but there are few data in humans. Some patients might need a short term blood transfusion programme in addition to the AED in order to improve oxygen delivery to poorly perfused tissue, although seizures may be precipitated by transfusion in certain contexts (Royal et al 1978, Siegel 1993) e.g. for priapism. More data is needed about the risks and benefits.

7.7. Conclusion

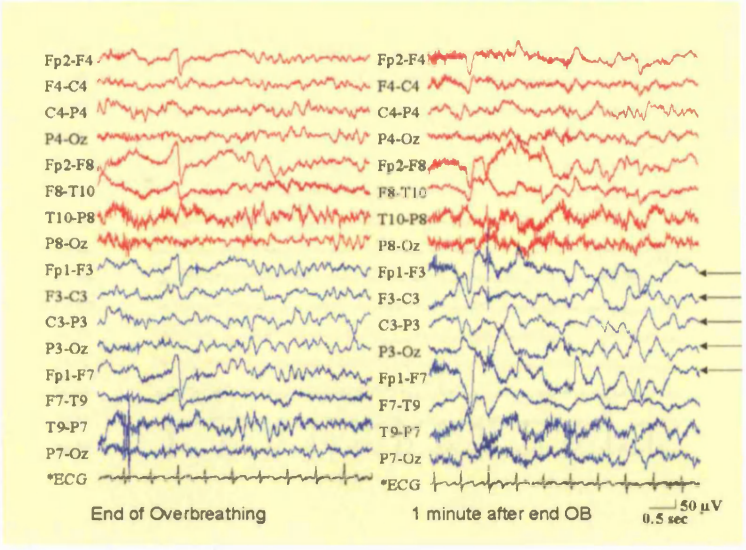
This study suggests that a complex mechanism of large and small vessel disease, hypoperfusion and perhaps chronic hypoxemia (Kirkham et al 2001a) may play a role in the genesis of seizures. In this study, daytime oxyhaemoglobin saturation was >96% in the majority of patients with seizures, but we did not have measurements from the time of the index seizures or overnight studies in this cohort or any comparison with the asymptomatic patients. However, it may be of interest that haemoglobin levels were higher in the seizures group than in the non-seizure symptomatic and the asymptomatic patients, although relative anemia is a risk factor for stroke in this population (Kirkham et al 2001a, Balkaran et al 1992).

SCD may serve as a model for hypoxia/ischaemia associated seizures, which might perhaps occur secondary to ion channel dysfunction during acute hypoxia in subjects who are poorly preconditioned (Stenzel-Poore et al 2003). This situation may be more common in young children than previously recognised, particularly in those exposed to anemic and hypoxic hypoxia, as in sickle cell disease. Further research will be necessary to study whether other genetic or environmental factors in addition to cerebral insufficiency, predispose to seizures in this population.



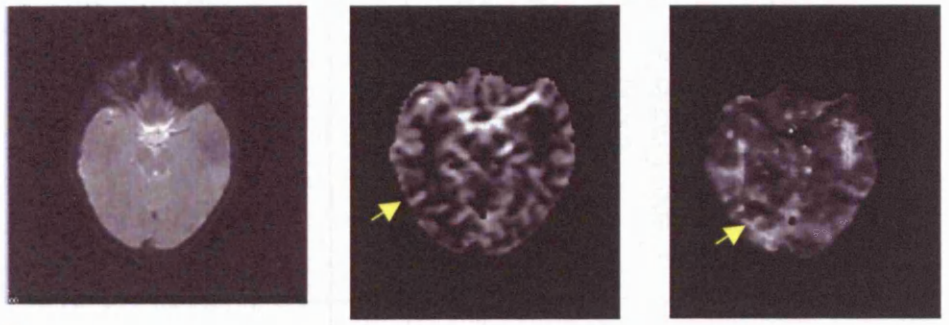
R L

MRI Perfusion MRI (MTT) MRA & TCD



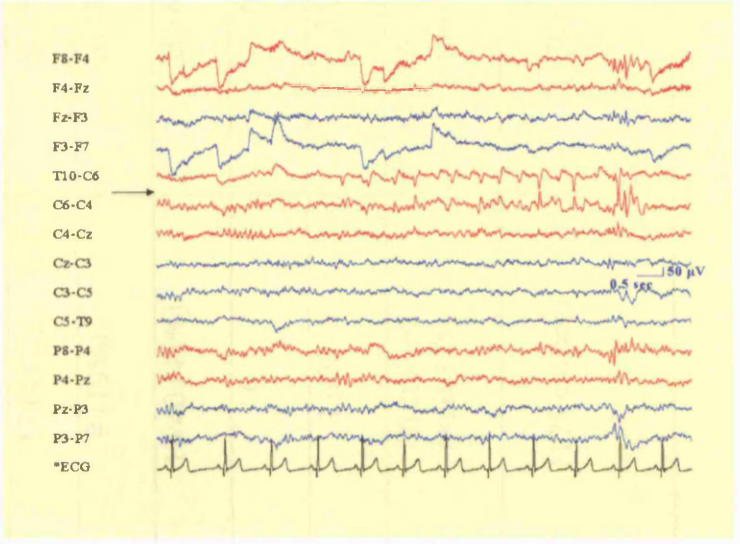
EEG

Figure 7.1. Seven-year old girl (HbSS). Seizures (facial twitching and blank spells) + stroke), TCD > 200 cm/sec, abnormal MRI (left precentral cortex infarct), MRA (reduced blood flow in the left middle cerebral artery), abnormal EEG (epileptic activity mainly over the left central-temporal regions [black arrows]) and Perfusion MRI (extensive cortical and sub-cortical perfusion abnormalities in the left frontal, parietal and temporal regions with severe decrease in cerebral blood flow, and increase in the mean transit time of the passage of Gadolinium bolus). R: right; L: left.



R L

MRI Perfusion MRI (CBF) Perfusion MRI (MTT)



EEG

Figure 7.2. 12-year-old boy with sickle cell β^0 thalassaemia. 'Petit mal epilepsy'. Normal MRI/MRA/TCD, abnormal EEG (runs of focal spikes over the right Sylvian area [black arrows]) and Perfusion MRI (mild decrease in cerebral blood flow and increase in the mean transit time over the right sylvian and posterior temporal areas on MRI perfusion [yellow arrows]). R: right; L

Table 7.1. Comparison between sickle cell patients with seizures, non-seizure neurological symptoms and no symptoms.

	SCD patients with seizures n=6	**Non-seizure neurologically symptomatic SCD patients n=41**	**Neurologically asymptomatic controls with SCD N=29**	**P for difference**
Abnormal MRI	3 (50%)	17 (41%)	10 (34%)	0.72, χ^2 test
Silent Infarct	2 (33%)	8 (19%)	10 (34%)	0.34, Fisher's exact test
Abnormal MRA	4 (67%)	19/40 (47%)	20 (69%)	0.18, χ^2 test
Abnormal DWI	0	0	0	
Abnormal TCD	5/6	11/29	5/24	0.005, χ^2 and Cramer's V tests
TCD mean maximum MCA velocities (range)	135 cm/sec (59-250)	96 cm/sec (<50-175)	120 cm/sec (66-176)	0.025, One way-ANOVA
Abnormal/Conditional TCD (Mean Mx MCA velocity ≥ 170 cm/sec)	2 (both >200 cm/sec)	1 (175 cm/sec)	1 (176 cm/sec)	0.006, χ^2 and Cramer's V tests

Table 7.1. Comparison between sickle cell patients with seizures, non-seizure neurological symptoms and no symptoms (cont.).

	SCD patients with seizures n=6	Non-seizure neurologically symptomatic SCD patients n=41	Neurologically asymptomatic controls with SCD N=29	P for difference
Median systolic blood pressure (range) (mmHg)	113 (100-115)	109 (94-129)		0.89
Median diastolic blood pressure (range) (mmHg)	66 (40-69)	55 (30-76)		0.64
Median mean arterial pressure (range) (mmHg)	84 (61-84)	73 (55-90)		0.59
Hemoglobin g/dL	9.3 (8.1-10.3)	8.7 (6.1- 10.4)	8.6 (7-14)	0.09, Kruskal-Wallis test
Oxyhemoglobin saturation (%)	97.2 (95.8 - 98.5)	95.4 (88.1 - 99.9)		0.1

SCD= sickle cell disease; TCD= transcranial Doppler ultrasound; MCA= middle cerebral artery; Mx: maximum

Table 7.2. Associations between focal EEG and neuroimaging / TCD findings in the sickle cell patients with seizures.

EEG/ Neuro-Imaging Relation	No, Sex, Age	Seizure Type	EEG	TCD (Max MCA_V)	Time from last seizure (last blood transfusion) to DSC-MRI (days)	MRI	MRA	Perfusion MRI	Therapy and outcome
Left Focus	Pat 1, F, 7 y HbSS, seizures, stroke (**right** hemi)	**Right** focal seizure at stroke onset. Follow-up: facial twitching and staring spells (atypical absences)	1st EEG (*2 mo after stroke*): Excess of slow activity over the **left** hemisphere. 2nd EEG (*1 y after stroke*): Isolated brief runs of sharp and spike wave over the **left** centro-temporal regions; occasional sharp waves over the right. 3rd EEG (*18 mo after stroke*): normal.	R: 137 cm/sec **L**: 250 cm/sec *After 1 year*: R: 121 cm/sec **L** : <50 cm/sec	7 days (29 days after and 1 day before blood transfusion)	**Left** precentral cortex infarct, further areas of high T2 signal in the peritri-gonal regions. *After 1 year*: no new infarcts. Cortical atrophy **left** MCA territory	Reduced flow in the proximal **left** MCA *After 1 year*: **L**.MCA narrow, poor distal flow, collaterals	No Perfusion *After 1 year*; Extensive perfusion abnormalities in cortical/subcortical **left** frontal, parietal and temporal regions.	Blood transfusion Seizure recurrence

EEG/ Neuro-Imaging Relation	No, Sex, Age, Diag.	Seizure Type	EEG	TCD (Max MCA_V)	Time from last seizure (last blood transfusion) to DSC-MRI (days)	MRI	MRA	Perfusion MRI	Therapy and outcome
Right Focus	Pat 2, F, 3 y, HbSS, seizure	Non-febrile **generalised** tonic seizure (one event)	1st EEG : No abnormality 2nd EEG (*after 1 year*): Frequent isolated spikes over the mid-parietal region over the **right**>left parietal and occipital areas.	1st TCD: R: 83 cm/sec L: 80 cm/sec 2nd TCD (*after 1 y*): R: 91 cm/sec L: 90 cm/sec	365 days No blood transfusion	Not done *After 1 y*: Normal	Not done *After 1 y*: Turbulent flow in the **right** M1 and A1 segments	*After 1 year*: Extensive perfusion abnormality in cortical/subcortical frontal-parietal regions bilaterally, cortical/subcortical temporal-occipital regions **right**>left and L. cerebellum.	None No seizure recurrence
Right Focus	Pat 3, F, 12 y HbSS, TIAs, seizures, headaches	**Right** sided tonic or jerking seizures of the arm and leg	Minor irregularities over the **right** central region.	**R**: 218 cm/sec L: 130 cm/sec *After 1 year*: **R**: 214 cm/sec L: 100 cm/sec	0 day (29 days after and 1 day before blood transfusion)	Tiny foci of abnormal signal in the deep white matter mainly on the **right** hemisphere.	**Right** proximal MCA stenosis	During R tonic seizure: Perfusion abnormalities in the **right** parietal – temporal deep white matter and **right** cortical /subcortical temporal region.	Blood transfusion + Carbamazepine (then changed to Lamotrigine due to cardiac arrythmia) Seizure recurrence

EEG/ Neuro-Imaging Relation	No, Sex, Age	Seizure Type	EEG	TCD (Max MCA_V)	Time from last seizure (last blood transfusion) to DSC-MRI (days)	MRI	MRA	Perfusion MRI	Therapy and outcome
Right **Focus**	Pat 4, M, 12 y, Sickle cell β^0 thal, seizures	Staring spells	Runs of focal spikes over the **right** sylvian area	R: 100 cm/sec L: 108 (Decreased **R** MCA velocities < 50 cm/sec below 4.4 cm depth)	30 days No blood transfusion	Normal	Normal	Perfusion abnormalities over the **right** sylvian area and posterior temporal lobe	Sodium Valproate Seizure recurrence
Left and Right Focuses	Pat 5, F, 21 y, HbSS, Seizures, stroke	Hallucinations, Visual phenomena	Excess of slow activity over the **left** temporal region with isolated sharp waves	**R**: <50cm/sec L: 59 cm/sec	7 days No blood transfusion	Prominence of cortical sulci, cerebral atrophy	Poorer flow in the **right** MCA	Abnormal perfusion in the parietal- temporal deep white matter, **right>left**	Sodium Valproate Seizure recurrence
No Focus	Pat 6, M, 12 y HbSS, severe SCD, seizures, behaviour problems, headaches	Atypical absences, **right** sided twitching, nocturnal seizures	Normal	R: 78 cm/sec L: 90 cm/sec (Turbulent blood flow US signal from **R** MCA)	210 days No blood transfusion	Normal	Normal	Perfusion abnormality in the **right** cortical/ subcortical temporal region	Sodium Valproate, Changed for Lamotrigine Seizure recurrence

Table 7.2. Abbreviations: Pat.= patient; BP = blood pressure; Dx = diagnoses; F = female; Hb = haemoglobin level (g/dL); HbSS = homozygous sickle cell anaemia; L = left; M = male; MAP= mean arterial pressure; Max MCA_V = maximum mean middle cerebral artery velocities; Mo = months; MRI/GA = MRI under general anaesthesia; No = patient number; R = right; SCD = sickle cell disease; TCD = transcranial Doppler Ultrasound; TIA = transient ischaemic attack; Tx = blood transfusion; y = year

Chapter 8: General Discussion

8.1. Cerebral Perfusion Abnormality in Sickle Cell Disease

This study has shown that an important proportion of patients with sickle cell disease and neurological complications had cerebral perfusion abnormalities. The abnormal perfusion may not change or may deteriorate over time despite blood transfusion or alternative therapies in an a significant number of sickle cell patients with central nervous system events, and the perfusion abnormality improved in only a small number of patients. Perfusion abnormality in these patients with sickle cell disease was significantly associated with abnormal magnetic resonance angiography (MRA), showing that the presence and progression of cerebrovascular disease (CVD) is a risk factor for cerebral ischaemia in this population. In addition, there was no reversal of the CVD in the majority of the patients who were on blood transfusion therapy. Blood transfusion does not appear to improve cerebral perfusion.

The aim of the cross-sectional study was to determine patterns of cerebral perfusion abnormality in relation to neurological symptoms in patients with SCD. The study showed that a large proportion of the patients with neurological complications had perfusion abnormalities. The patterns of the perfusion abnormality, including the severity and the extension of the abnormal perfusion, were associated with the severity of the central nervous system events at presentation and the recurrent neurological symptoms of the patients.

In addition, this cross-sectional study showed that, although there was a good association between the severity of the abnormal perfusion and the abnormalities found in MRI, MRA and transcranial Doppler ultrasound, perfusion MRI (dynamic susceptibility contrast MRI [DSC-MRI]) was the most sensitive technique to detect cerebral abnormality (means by abnormal perfusion) in 56% of the 70 patients with SCD of this study compared to MRA (46%) and MRI (40%).

In relation to previous cross-sectional studies using perfusion MR techniques such as DSC-MRI (Kirkham et al 2001) or continuous arterial spin labelling (ASL, Oguz et al 2003), this study focused on identifying patterns of cerebral perfusion abnormality in relation to neurological symptoms in patients with SCD and any association between grades of abnormality and conventional neuroimaging (MRI and MRA) or transcranial Doppler ultrasound studies (chapter 7 is a detailed study of one neurological symptom, seizures in SCD, and its association with cerebrovascular disease and cerebral perfusion abnormality). In addition, predictors of recurrent neurological symptoms were identified from a single time study. The sensitivity of perfusion MRI may give useful information to the clinician for those patients with SCD who are at serious risk of ischaemia and further stroke (overt infarct) or covert infarction, two neurological complications which can increase, in the different grades of severity, the disability of the patient.

Cerebral perfusion abnormality in relation to age groups showed that abnormal perfusion was more extensive (bilateral) in symptomatic patients less than 5 years old who had predominantly normal MRI and MRA. This finding might suggest that the developing brain is most sensitive at the level of small blood vessels or microcirculation to the abnormal rheology of the sickle cell probably triggered by infections or decreased Oxygen saturations (e.g. respiratory infections, obstructive sleep apnoea [Kirkham et al 2001a, Hogan et al 2003]). A recent study in babies with SCD showed an association between abnormal Oxygen saturations, TCD and cognitive development (Hogan et al 2003). The finding presented in this thesis of widespread abnormal perfusion in patients with neurological symptoms in this age group confirms that SCD may cause cerebral pathology in very young children, as has been reported previously in a small series investigated only with MRI and MRA studies (Wang et al 1998). Therefore the presence of abnormal perfusion in sickle cell children of this age may guide the development of early preventive treatment for very young patients with SCD who are at risk of cerebral ischaemia, in order to avoid more severe complications over time. On the other hand, cerebral perfusion abnormality was more localised from 6 years of age and beyond, and the abnormal perfusion was related to the vascular territory involved by the cerebrovascular disease. Further studies could explore the adaptable changes of the cerebral microcirculation over time and in relation to brain development in SCD.

The longitudinal study demonstrated than more than a third of the patients had progression of the perfusion abnormality over time, and this progression continued, in many cases, despite chronic blood transfusion. This progression of the abnormal cerebral perfusion was associated with progression of cerebrovascular disease, and the severity of the perfusion abnormality over time was related to the severity of the recurrent neurological symptoms of the patients. On the other side, another third of the patients had unchanged cerebral perfusion, with mainly unchanged MRI and MRA, with or without blood transfusion therapy. Only a small proportion of patients had an improvement of the cerebral perfusion over time. This is the first study of changes in cerebral perfusion over time in patients with SCD and neurological complications with long-term follow-up. Previous studies have only reported longitudinally changes using only MRI and/or MRA investigations in patients with SCD (Moser et al 1996, Kandeel et al 1996, Seibert et al 1998, Miller et al 2001, Pegelow et al 2002), or short-term (pre- and post-blood transfusion) studies using techniques which measure cerebral blood flow (Hurlet-Jensen et al 1994, Venketasubramanian et al 1994, Kirkham et al 2001b). Perfusion MRI was the most sensitive technique to evaluate cerebral abnormality in relation to neurological symptoms in this study and changes over time, in association with MRA abnormalities.

The longitudinal study could also identify predictors of recurrent neurological symptoms and abnormal MRI, MRA and perfusion abnormalities at follow-up by comparing initial and final investigations. There was a good correlation among initial infarct size, infarct number, perfusion abnormality (CBF) and grade of MRA turbulence; and the severity of the worsening of the cerebral perfusion at follow-up was related to the severity of the initial neurological symptoms at presentation and the abnormality of the initial investigations. Because there is a good correlation between the initial investigations, cerebral perfusion at follow-up could be predicted with one or two MR studies in addition to the neurological symptoms of the patients, and to identify predictors could give additional information to the clinician for the future management of the patient depending on his grade of risk of further cerebral ischaemia.

The study of the effect of blood transfusion therapy on the cerebral perfusion showed that in the short-term (pre- and post-blood transfusion) there was an improvement on the cerebral perfusion in more than half of the patients after blood transfusion, with

unchanged MRI and mainly unchanged or worse MRA. This study demonstrated that blood transfusion might increase turbulence in the cerebral blood vessels in some patients with extensive (or not) cerebrovascular disease without worsening of the perfusion abnormality; therefore MRA was not a sensitive technique for evaluating perfusion changes after transfusion in these patients with SCD. However, this study agreed with previous series using cerebral blood flow techniques (Hurlet-Jensen et al 1994, Venketasubramanian et al 1994, Kirkham et al 2001b), that short-term blood transfusion can be beneficial for improving perfusion abnormality.

In the long term, although blood transfusion helped to reduce recurrence of stroke in these patients (in only one patient had stroke recurrence out of the 17), it did not stop the progression of cerebrovascular disease and the perfusion abnormality in nearly half of the patients. However, serial haemoglobin S% levels were not available for the longitudinal study or blood transfusion studies (for example, at the time of the first or final scans or before the blood transfusion started). This issue should be addressed in future work. In addition most of the patients continued to have recurrent neurological symptoms, including those with unchanged or improved perfusion abnormality. This study showed that blood transfusion is effective in preventing recurrence of severe neurological complications in patients with SCD but this therapy was not effective for reversal of cerebrovascular disease and cerebral perfusion abnormality in the long term, therefore these sickle cell patients continued to be at risk of further cerebral ischaemia and infarction. The direct effect of blood transfusion, such as iron overload and iron toxicity, on the cerebral vasculature has been reported (Day et al 2003, Schmitz 2003, Shah and Alam 2003, Kuang et al 2003). Alternative therapies could be considered of benefit for selected patients, especially those patients with poor compliance for the blood transfusion regime or those who had iron overload, in addition with an underlying cerebrovascular disease. Perfusion MRI (DSC-MRI) can be used to study alternative therapies such as Hydroxyurea, bone marrow transplantation and revascularisation procedures and their effect on cerebral perfusion. One patient of this study, who had an indirect revascularisation, showed an improvement in the cerebral perfusion. Revascularisation could be considered in those patients with SCD and severe and irreversible cerebrovascular disease and severe recurrent neurological symptoms.

The categories of severity of the transcranial Doppler ultrasound study, following a modification of Adams' criteria (Adams et al 1992, Kirkham et al 2001b) were associated with grades of abnormal perfusion, MRA abnormality and infarct size and infarct number on MRI in this study, demonstrating the importance of this non-invasive technique as a tool for monitoring not only cerebrovascular disease in patients with SCD (Adams et al 1997, Adams et al 1998, Adams et al 2004) but also to monitor cerebral perfusion, and probably to detect early cerebrovascular disease (Minniti et al 2004) before it appears on MRA. In addition, TCD could help to predict recurrent neurological symptoms in sickle cell patients, because its association with MRI, MRA and perfusion abnormalities.

In regard to clinical parameters, lower diastolic blood pressure and mean arterial blood pressure (MAP) were associated with the presence of cerebral infarction on MRI, abnormal MRA and abnormal perfusion in the cross- sectional study. In the longitudinal study, there was a trend for an association between lower diastolic blood pressure and worsening of the transcranial Doppler ultrasound over time. Although 'relative' hypertension has been considered a risk factor for stroke in SCD (Ohene-Frempong et al 1991, Rodger et al 1993), and low blood pressure values have been reported in patients with SCD (Pegelow et al 1997), the association of low diastolic pressure and MAP with perfusion abnormality in this study suggests that a decrease in blood pressure and, probably, blood flow to the brain, might be a contributing factor to impairment of perfusion, especially in older patients.

In the longitudinal study, there were trends for association between worsening of cerebral perfusion over time and worsening of MRI with increased white cell count and lymphocytes over time. In addition there was an association between decreased initial platelet count and worsening of MRA over time. These findings suggest the involvement of infection in the progression of cerebrovascular disease and the worsening of the cerebral perfusion over time. The damaging effect of infectious/inflammatory mechanisms in blood vessels was reported in previous studies (Sultana et al 198, Belcher et al 2000, Inwald et al 2000).

8.2. Limitations of the Study

The timing of the investigations was a limitation in this study because the majority of the patients were studied as outpatients and not acutely, with the exception of a few cases. Therefore the real extent and the severity of the perfusion abnormality (or the presence of perfusion abnormality) in relation to the neurological symptoms at presentation were difficult to assess. In fact, some older patients had had stroke years before to this study, however, the neurological event at presentation was taken in account because of the persistence of the abnormal perfusion in the territory of the ischaemic lesion, the presence of cerebrovascular disease and recurrent neurological symptoms, which were related to the initial infarct on MRI perfusion abnormality present in a proportion of the patients of those age groups (13-17 years and 18-28 years).

In this study, there were limitations in the analysis of MRI, MRA and perfusion MRI, because of the visual assessment of these investigations. As was mentioned in chapter 4 and chapter 5 (in the 'Discussion' section), there were, in a very few occasions, small discrepancies in the assessment of the grade of turbulence on MRA by the same Neuroradiologist (intra-observer), when the MRA study was assessed twice by the same specialist. On the other hand, discrepancies on the assessment of MRI studies were minimal.

Dynamic susceptibility contrast (DSC) perfusion MRI was a sensitive technique to detect cerebral abnormality, however it also had limitations. Firstly, it was not a quantitative technique, so it was not possible to measure cerebral blood flow and to assess real changes of cerebral blood flow volume over time, or to quantify the severity of the perfusion abnormality in a cerebral region. Another perfusion technique used in patients with SCD, continuous arterial spin labelling (ASL), which does not need an intravenous bolus injection of Gadolinium, is able to quantify cerebral blood flow (Oguz et al 2003), however this technique is still under development and there are technical limitations for the exact measurement of CBF. On the other hand, the assessment of perfusion MRI is visual (Calamante et al 1999) and therefore the data for

analysis were qualitative (ordinal data) based on a grading or categories of perfusion abnormality of increasing severity (either for an increase in the mean transit time (MTT) of the passage of the intravenous bolus of the contrast through the cerebral vasculature, or for a decrease in the CBF). The author, supervised by a Physicist experienced in this MR technique, assessed the MR perfusion maps (MTT, CBF, and cerebral blood volume [CBV]) of all the patients (n=70). As was mentioned in chapter 4 and 5, the grading of the CBF maps (mild, moderate, or severe decrease in CBF) presented a more significant association with neurological symptoms and other investigations (neuroimaging and TCD) than the grade of severity of MTT (mild, moderate or severe increase in MTT). This may happen because the MTT appears more highlighted on the visual inspection as MTT is white and CBF appears dark on the perfusion MRI maps, therefore it was more difficult on the visual assessment to differentiate grades of severity on the MTT maps than on the CBF. From this study CBF maps were the most sensitive parameter to assess perfusion abnormality.

Transcranial Doppler ultrasound (TCD) was a very useful non- invasive technique in this study, in agreement with previous studies (Adams et al 1992, Adams et al 1998). In addition categories of severity of the TCD (following a modification of Adams'criteria, Adams et al 1992, Kirkham et al 2001b) had a good correlation with grades of abnormal MRA and abnormal perfusion. However this technique had a limitation in this study in relation to the sensitivity to detect cerebrovascular disease based especially on the ultrasound window (e.g. thick skull secondary to bone marrow changes), which affected mainly the older age group of patients (mainly 18-28 years of age, and a smaller proportion of patients of 13-17 years of age) giving rise to false positive results (abnormal TCD with normal MRA). In fact, in this case, TCD might be more sensitive than MRA to detect early cerebrovascular disease (Minniti et al 2004), as some of these patients continued having recurrent neurological symptoms and abnormal perfusion. Another limitation was the TCD operator; although the author had experience in this technique, on some occasions the TCD study was more difficult to do in one patient than in another.

The limitation of the haematological data was the availability to find blood tests taken to the patients near to their neuroimaging study in their clinical records, and the availability to find the patients' clinical records. In addition, steady haemoglobin levels were not easy to find, as usually the patients had their blood test taken on the ward more than as out- patients, when they had sickle cell crises, sometimes complicated by infections of different grade of severity, or the blood test were taken before or after transfusion (pre-transfusional blood test were difficult to find usually) in those patients on chronic blood transfusions. In the extension of this study and in future studies, the documentation of haemoglobin S% level at the time of the neuroimaging and any other central nervous system event will be crucial.

In this study, only awake-Oxygen saturation (SpO_2) was measured and it did not have association with neurological symptoms and other investigations. Nocturnal hypoxemia was associated with central nervous system events in SCD (Davies et al 1989, Kirkham et al 2001a), and awake- SpO_2 was not a good measure of disease severity in this study, probably because the small numbers of patients who had recurrent stroke in this series, these patients had SpO_2 < 92% defined as hypoxemia for this study. Awake- SpO_2 < 90% was not associated with stroke risk in a previous series of patients with SCD (Homi et al 1997). Nocturnal hypoxemia and nocturnal SpO_2 measurements might be better predictors of neurological symptoms and abnormal investigations in SCD as was reported before (Kirkham et al 2001a).

8.3. Future Directions

This study showed that perfusion MRI (DSC-MRI) was the most sensitive technique to detect cerebral abnormality in patients with sickle cell disease, giving information about perfusion abnormality and therefore perhaps about the risk of cerebral ischaemia in an individual. However, data from more patients with longer follow-up will be needed to determine whether MR perfusion is more useful than clinical criteria for predicting recurrence. This technique was well tolerated by the majority of the patients and few patients (3% [2/70 patients]) had side effects, which were mild and transient. Perfusion MRI could help to guide and monitor the management of a patient, especially in those

patient who have severe recurrent neurological symptoms or poor compliance with the blood transfusion therapy.

In addition a single study of perfusion MRI, together with conventional neuroimaging (MRI and MRA) and TCD, could help to predict in a patient with SCD who had an initial central nervous system event, recurrent neurological symptoms and perfusion abnormality at follow-up, leading to individually tailored management to prevent cerebral ischaemia and further disability.

Perfusion MRI could also be used to assess the benefit of new treatments for patients with SCD and neurological complications on cerebral perfusion and to monitor these new therapies over time (i.e. Hydroxyurea).

In addition, perfusion MRI may be combined with other novel non-invasive MR techniques, such as BOLD hyperoxia (Kennan et al 2004) to investigate the pathophysiology of CNS events in sickle cell disease. The author has undertaken a pilot demonstrating that this is feasible. As central nervous system events have been associated with nocturnal hypoxemia (Kirkham et al 2001a) perfusion and BOLD MRI could be used to assess the effect of nocturnal low oxygen saturations on cerebral perfusion in patients with SCD and to monitor the effect of treatments under investigation in the setting of randomised controlled trials, such as overnight oxygen supplementation in patients with nocturnal oxyhaemoglobin desaturation.

In summary, although perfusion MRI is a semiquantitative technique, it has considerable potential, in combination with other neuroimaging techniques, to shed light on the natural history and pathophysiology of the cerebral complications of sickle cell disease.

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Appendix

Table 1. Chapter 2: Sickle cell patients of this study (n=70). S= stroke; Z=seizures; N=none; C=coma; H=headaches; L= learning difficulty; AT= anterior territory TIA; PT=posterior TIA; R=reversible ischaemic neurological deficit (RIND); T= transient ischaemic attack (TIA), presymp= neurological symptom at presentation; recsymp= recurrent neurological symptom; TCD=transcranial Doppler ultrasound; perf.= perfusion MRI; datprvpf= date of previous perfusion MRI study; mrifup= MRI at follow-up; Txdate= date of blood transfusion; perfptx= perfusion MRI for the pre-and post-transfusion study; postday= date of post-transfusion MRI.

PAT	SEX	AGE	PRESYMP	RECSYMP	DATETCD	DATEMRI	DWI	MRA	PERF	DATPRVPF	MRIFUP	TXDATE	PERFPTX	POSTXDAY
1	F	4	S,Z	N	21-Jan-2003	22-Jan-2003	Yes	Yes	Yes					
2	M	12	S,C,H,L	PL	11-Jul-2002		Yes	Yes	Yes	30-Nov-1999				
3	F	7	S,Z,L	Z,L	12-Sep-2001	12-Jun-2003	Yes	Yes	Yes	12-Sep-2002	12-Jun-2003		No	
4	F	28	S	N	23-Nov-2001	23-Nov-2001	Yes	Yes	Yes					
5	F	16	S,AT,H,L	H,L	12-Oct-2001	12-Oct-2001	Yes	Yes	Yes	18-Dec-1998	10-Jul-2003	19/10/01	Yes	25/10/01
6	M	16	S,C,H,L	L, H		15-Aug-2001	Yes	Yes	Yes	25-Aug-1999	!	22/06/01	Yes	04/07/01
7	F	19	S,R,AT,PT,H,L	T, H, L	04-Jul-2002	04-Jul-2002		Yes	Yes	12-Feb-2002	06-Sep-2002			
8	F	16	S,AT,Z,H,L	T, Z, H, L	17-Jun-2002	19-Jun-2002	Yes	Yes	Yes	29-Jun-1999		20/06/02	Yes	24/06/02
9	F	27	S	N	12-Mar-2002	12-Mar-2002	Yes	Yes	Yes			12/04/02	Yes	12/04/02
10	F	15	S,Z,H,L	Z, H, L	19-Oct-2001		Yes	Yes	Yes			26/10/01	Yes	02/11/01
11	M	8	S,AT,H,L	T, H, L	06-Dec-2001	06-Dec-2001	Yes	Yes	Yes		06-Mar-2003	30/11/01		
12	F	24	S,AT,H,L	T, H, L	15-Nov-2001	15-Nov-2001	Yes	Yes	Yes	18-Jun-1999				
13	M	16	S,PT,H	T, H	14-Mar-2002	14-Mar-2002	Yes	Yes	Yes	17-Sep-1999		16/03/02	Yes	21/03/02
14	F	10	S,L	L	28-Feb-2003	28-Feb-2003	Yes	Yes	Yes					
15	M	22	S,Z,H,L	S, Z, L	10-Apr-2003	10-Jul-2003	Yes	Yes	Yes			11/04/03	Yes	17/04/03
16	M	13	S	T	11-Jul-2001	11-Jul-2001	Yes	Yes	Yes	02-Jul-1999				
17	F	1	AT	N	29-Jan-2003	09-Dec-2002	Yes	Yes	Yes					
18	M	16	AT	N	13-Dec-2001	13-Dec-2001	Yes	Yes	Yes		25-Jul-2003	15/12/01	Yes	20/12/01
19	F	25	AT,H,L	T, H, L	07-Dec-2001		Yes	Yes	Yes	23-Nov-1999				
20	F	11	AT,Z,H	T, Z, H	31-Aug-2001	03-Sep-2001	Yes	Yes	Yes	30-Nov-1999	19-Jun-2003	03/01/02	Yes	04/01/02
21	M	9	AT	T	23-Nov-2001	26-Nov-2001	Yes	Yes	Yes					
22	M	15	AT,H,L	T,H,L	29-Aug-2002	29-Aug-2002	Yes	Yes	Yes	18-Jun-1999				
23	M	18	AT,H	H	18-Jul-2002		Yes	Yes	Yes					
24	F	22	AT,H	H	23-Jan-2003		Yes	Yes	Yes					
25	M	1	AT	N	06-Mar-2003	07-Mar-2003	Yes	Yes	Yes					
26	M	16	PT,H	H	14-Nov-2001	14-Nov-2001	Yes	Yes	Yes					
27	M	15	PT,H,L	H, L	13-Feb-2002	13-Feb-2002	Yes	Yes	Yes	18-Jun-1999				
28	M	16	PT,H	T, H	23-Aug-2001	23-Aug-2001	Yes	Yes	Yes					

PAT	SEX	AGE	PRESYMP	RECSYMP	DATETCD	DATEMRI	DWI	MRA	PERF	DATPRVPF	MRIFUP	TXDATE	PERFPTX	POSTXDAY
29	M	19	PT,H	H	04-Feb-2002	04-Feb-2002	Yes	Yes	Yes	30-Jul-1999				
30	M	15	PT,H	H	24-Mar-2003	24-Mar-2003	Yes	Yes	Yes			26/03/2002	Yes	27/03/2003
31	F	19	PT,C,Z	H	19-Jun-2001	19-Jun-2001	Yes	Yes	Yes	24-Mar-1999				
32	F	16	PT,L	L	19-Oct-2001		Yes	Yes	Yes			26/10/01	Yes	02/11/01
33	M	10	PT,H,L	H, L	29-Nov-2001	29-Nov-2001	Yes	Yes	Yes					
34	F	6	PT,H,L	H,L		07-Nov-2002	Yes	Yes	Yes					
35	M	10	PT	N	07-Mar-2002	07-Mar-2002	Yes	Yes	Yes					
36	F	16	PT,H	H, L	08-Nov-2001	08-Nov-2001	Yes	Yes	Yes					
37	M	12	PT,Z,H,L	Z, H, L	21-Feb-2002		Yes	Yes	Yes					
38	F	13	PT,H,L	T, H, L	12-Nov-2001	12-Nov-2001	Yes	Yes	Yes	30-Jul-1999				
39	M	13	PT,H,L	H, L	26-Sep-2002		Yes	Yes	Yes					
40	M	16	PT,H	T, H	15-Feb-2002		Yes	Yes	Yes					
41	M	11	PT,C,H,L	T, H, L		07-Dec-2000	Yes	Yes	Yes					
42	F	21	Z,L	Z, L	24-Jan-2002	24-Jan-2002	Yes	Yes	Yes	01-May-2001				
43	M	7	Z,L	L, Z	31-Oct-2002	31-Oct-2002	Yes	Yes	Yes					
44	F	2	Z	N	14-Mar-2002	14-Feb-2003	Yes	Yes	Yes		14-Feb-2003			
45	M	13	Z	Z	17-Aug-2001	17-Aug-2001	Yes	Yes	Yes					
46	M	4	Z,L	Z, L			Yes	Yes	Yes					
47	1	17	Z	H	03-Jul-2001	03-Jul-2001	Yes	Yes	Yes	01-Sep-1999				
48	0	12	Z,H,L	Z, H, L	26-Oct-2001	26-Oct-2001	Yes	Yes	Yes					
49	1	11	H,L	H,L	07-Mar-2002	07-Mar-2002	Yes	Yes	Yes	17-Jun-1999				
50	0	24	H	H	07-Nov-2001	07-Nov-2001	Yes	Yes	Yes	24-Mar-2000				
51	0	5	H	H	31-Oct-2002	05-Feb-2003	Yes	Yes	Yes					
52	0	15	H,L	T, H, L	29-Aug-2002	#NULL!	Yes	Yes	Yes	24-Feb-1999				
53	0	22	H, L	H,L	22-Oct-2001	22-Oct-2001	Yes	Yes	Yes					
54	1	16	H.L	H, L	10-Oct-2001	#NULL!	Yes	Yes	Yes	06-Jul-1999				
55	0	9	H,L	H, L	14-Feb-2002	14-Feb-2002	Yes	Yes	Yes			18/02/02	Yes-movement-	21/02/02
56	0	21	H	N	13-Jul-1979	13-Jul-2001	Yes	Yes	Yes					

	SEX	AGE	PRESYMP	RECSYMP	DATETCD	DATEMRI	DWI	MRA	PERF	DATPRVPF	MRIFUP	TXDATE	PERFPTX	POSTXDAY
57	0	11	H,L	H,L	20-Jul-2001	20-Jul-2001	Yes	Yes	Yes	30-Jun-1999				
58	1	24	H	H, L	09-Nov-2001	09-Nov-2001	Yes	Yes	Yes					
59	0	9	H	H	21-Sep-2001	21-Sep-2001	Yes	Yes	Yes					
60	1	9	H, L	H, L	12-Jul-2002		Yes	Yes	Yes					
61	1	14	H	H	08-Feb-2002	08-Feb-2002	Yes	Yes	Yes					
62	1	11	H,L	H, L	07-Feb-2002	07-Feb-2002	Yes	Yes	Yes					
63	0	20	H	H	01-Oct-2001	01-Oct-2001	Yes	Yes	Yes	02-Jul-1999				
64	0	11	L	L	19-Aug-2002		Yes	Yes	Yes					
65	0	4	L	L			Yes	Yes	yes					
66	1	10	L	L	05-Sep-2002		Yes	Yes	Yes					
67	0	13	L	L	08-Nov-2001	08-Nov-2001	Yes	Yes	Yes					
68	1	8	N	N	31-Aug-2001	03-Sep-2001	Yes	Yes	Yes					
69	1	10	N	L	05-Dec-2002	05-Dec-2002	Yes	Yes	Yes					
70	1	8	N	N	15-Mar-2002		Yes	Yes	Yes					

Table 2: Chapter 4

Table 2. Cross-sectional study (n=70 patients). Association between patients, diagnosis, haemoglobin level, awake-Oxygen saturation, neurological symptoms, MR studies and TCD. (chapter 4).

No, Sex, Age, Diagnosis, Haemoglobin (g/dl), SpO_2	Problem and *Recurrent Symptoms*	Main Symptoms at Onset	Rx	R TCD	R MRA	R MRI	R Perf.	L TCD	L MRA	L MRI	L Perf.
1.M,12 y, SS, 9.8, 98.3%	**C, S** *Coma (PLKE) + S, H*	Coma + R. hemi	Tx	0	0	FPT (DWM)	F**P**T**	3	0	FP (DWM)	F** P**
2.M, 16y, SS, 9.2, no SpO_2	**C, S** *H, L*	Coma, bilateral stroke, ICP	Tx SxD	4 ?win-dow	2	FPT	F*P*T*	4 ?win-dow	2	F	N
3.F, 19y, SS, no Hb, 96.7 %	**C,PT** *H*	Coma, H, dizzy, blurred vision		0	0	N	N	3	0	N	N
4.M, 13y, SS, no Hb, 89.9 %	**C,PT** *T, H, L*	C, T (facial weakness)	Tx	No data	0	N	N	No data	0	N	N
5.F, 4y, SS, no Hb, 98%	**S, Sz** *A*	R hemi + hemianop + R focal Sz	Tx	0	1	FP (+ DWM)	F**P**O**	4	1	FPT	F** P** T**
6. F, 7y, SS, 9.7, 97%	**S, Sz** *Sz, L*	R hemi + R focal Sz	Tx	0	0	FPT (DWM)	F**P**T**	3	4	FPT	F** P** T**
7. F, 27y, SC, no Hb, 99.9%	**S** *A*	R hemi or ?MS attack		0	0	FPT (SC)	T** (SC)	0	0	FPT (SC)	T* (SC)
8.F, 16y, SS, no Hb, 98.3%	**S, H** *H, L*	R hemi +H	Tx	3	1	N	N	3	4	FPT	F** P** T**
9. F, 19y, SS, 9.3, 96%	**S** *R, T, H, L*	L hemi	Tx stopped	4	3	F (+ DWM)	F**P**T**	3	2	FP (DWM)	F** P** T** (SC)
10.F, 16y, SS, no Hb, 97%	**S, Sz** *AT, H, L*	R hemi+ Sz+Aphasia	Tx	3	1	T	F**	3	4	FPT	F** P* T**
11.F, 27y, SS, 9.3, 97%	**S** *A*	Sz, collapse	Tx	0	1	PO	P**O*	0	3	PO	P*O*
12.F, 14y, SS, 8.8, 91.9%	**S** *H, L*	Learning Difficulty	Tx	0	1	N	F**P**T** O**	0	1	FP (+ DWM)	F** P** T** O**
13.F, 7y, SS, 10.9, 97.5%	**S** *AT, H, L*	L hemip	Tx	4	3	FPT (DWM)	F**P**T**	3	2	F (DWM)	N
14.F, 24y, SS, 8.4, 92.5%	**S** *S, T, H, L*	R & L hemi, dysphasia	Tx stopped	4	5 MM	FPTO (DWM)	F**P**T** O*	4	3 MM	FPTO	F*P* T** O*
15.M, 16y, SS, 10.1, 97%	**S, H** *H*	H, collapse, R hemianop	Tx	4	5 MM	FPT	P**T*O**	3	5 MM	O T(BG)	P** O**
16.F, 11y, SS, no Hb, no SpO_2	**S** *L*	Multiple strokes, R & L hemi	Tx stopped BMT	3	5 MM	F (+ DWM)	F**P**T**	3	5 MM	FP (+ DWM)	F** P** T**
17.M, 22y, SS, no Hb, 85%	**S** *S, H, L*	Onset L. hemi, recurrent L. hemi + Sz	Tx AED	4	4	FPT	F*P**T**	4	0	N	N
18.M, 10y, SS, no Hb, 97%	**S** *AT*	R hemi + Aphasia	Tx Stopped-BMT	3	3	FP (DWM)	F*P*T*O**	3	3	FP	F*P* T** O**
19.F, 9m, SS, no Hb,no SpO_2	**AT** *A*	?Transient L.leg paresis Dactylitis	ATB	0	0	N	P**T**O**	0	0	N	P** T** O**
20.M, 15y, SS, no Hb, 97%	**AT** *A*	TIAs, intracranial anerysms	Tx	3	1	N	N	3	N	FP (DWM)	N

No, Sex, Age, Diagnosis, Haemoglobin (g/dl), SpO_2	Problem	Main Symptoms at Onset	Rx	R TCD	R MRA	R MRI	R Perf.	L TCD	L MRA	L MRI	L Perf.
21.F, 25y, SS, 6.1, 97.9%	**AT** *AT, H, L*	Transient hemip + H	Tx stopped	4	2	FT (DWM +BG)	F*P**T* (SC)	4	1	FPT (DWM + BG)	P* T** (SC)
22.F, 9y, SS, 8.1, 92.5%	**AT** *AT, Sz, H*	Transient L & R hemi, R Sz, H, MCA veloc. >200 cm/s	Tx AED	2	5 MM	FPT (DWM)	F**P**T**	0	2	N	N
23.M, 8y, SS, 8.7, 97.6%	**AT** *AT*	TIA		0	Not done	N	N	0	Not done	N	N
24.M, 15y, SS, 7.8, no SpO_2	**AT** *AT, H, L*	Transient R hemip + H	Tx stopped LRV+HU	0	2	FPTO (BDZ)	F**P**T** O**	3	1	FPT (BDZ)	F** P** T** O**
25.M, 18y, SS, no Hb, 97%	**AT** *H*	R paraesthesia + R sided H		3	0	N	N	4	0	N	N
26.F, 22y, SS, no Hb, no SpO_2	**AT, H** *H*	H +L sided paraesthesia (pain)		3	0	N	P**	0	0	N	N
27.M,11m,SS, 9.6, no SpO_2	**AT** *A*	Transient L hemi	Tx	0	0	N	F**P**T** O** + cerebellum	0	0	N	F** P** T** O**+ cereb.
28.M, 15y, SS, no Hb, 95.5%	**PT** *H*	Transient L hemi + unconscious-ness + H		4	0	N	N	3	0	N	N
29.M, 16y, SS, 6.6, 88.9%	**PT** *H, L*	Headaches + Hallucinations	Tx stopped	0	0	N	N	0	0	N	N
30.M, 17y, SS, no Hb, 97.3%	**PT** *T, H*	H, dizziness, paraesthesia, confusion		0	0	N	N	3	0	N	N
31.M, 18y, SS, no Hb, 95.2%	**PT** *H*	TIA + headaches	Tx stopped-HU	3	0	N	T**	0	0	N	N
32.M, 16y, SS, no Hb, 92%	**PT** *H*	Collapse + transient loss of vision	Tx + Oxygen overnight	3	0	F (DWM)	F*P**	0	1	F (DWM)	F** P**
33.F, 15y, SS, 8.8, 93.5%	**PT** *H*	TIA + headaches	Tx	3	5 MM	FT (DWM + BG)	F**P**T** O**	3	0	T (BG)	F** P** T** O** (SC)
34.M, 9y, SS, 7.7, 96%	**PT** *H, L*	Headaches + pica + L		0	0	N	N	0	0	N	T** O**
35.F, 6y, SS, 6.9, 92%	**PT,H** *H, L*	Headaches + double vision + MCA veloc > 200cm/sec	Tx	0	3	N	F**P** (SC)	2	3	N	F** P** (SC)
36.M, 9y, SS, 5.7, 97.7%	**PT** *A*	Transient L hemi & unres-ponsiveness + blurred vision episodes	Acute Tx Aspirin	0	0	N	N	3	0	N	N
37.F, 15y, SS, 11, 97.8%	**PT** *H, L*	TIA + headaches		0	0	N	N	0	0	N	N
38.M, 12y, SS, 8.1, 95.9%	**PT, Sz** *Sz, H, L*	Blank episodes, R sided Sz + H	AED	0	0	N	T**	0	0	N	N

No, Sex, Age, Diagnosis, Haemoglobin (g/dl), SpO_2	Problem	Main Symptoms at Onset	Rx	R TCD	R MRA	R MRI	R Perf.	L TCD	L MRA	L MRI	L Perf.
39.F,13y, SS, 6.6, 95.7%	**PT, H** *T, H, L*	Transient hemi + VI nerve palsy + H	Tx stopped	4	0	FP (DWM)	N	4	1	PTO (DWM)	N
40.M, 13y, SS, 9, no SpO_2	**PT** *H, L*	Headaches + blurred vision		0	2 (PCA)	F (DWM)	N	3	1 (PCA)	F (DWM)	N
41.M, 11y, SS, no Hb, 94.8%	**PT, H** *T, H*	Headaches + dizziness		0	1	N	N	3	0	N	N
42.F, 20y, SS, 9.8, 98%	**Sz** *Sz ,L*	SCD crisis with ?stroke + hallucinations + visual phenomena	Acute Tx on AED	4	0	Mild atrophy	F**P**T** (SC)	3	0	Mild atrophy	F** P** T** (SC)
43.M, 8y, SS, 11.6, no SpO_2	**Sz** *Sz, L*	?blank spells + L		0	0	N	F**P**	0	0	N	P**
44.F, 20m, SS, 8.2, 98.7%	**Sz** *A*	Generalised tonic- clonic seizure		0	1	N	F**P**T** O**	0	1	N	F** P** T** O** Cereb.
45.M, 12y, S/B^0thalass, no Hb, 97%	**Sz** *Sz*	Staring spells (petit mal like)	AED	3	0	N	T**O**	0	0	N	N
46.M, 4y, SS, 9.2, no SpO_2	**Sz** *Sz, L*	Autism, ?blank spells		0	0	N	N	0	0	N	N
47.F,17y, S/B^0thalass, no Hb, 92.8%	**Sz** *H*	Seizures in infancy + occipital headaches		0	0	N	N	0	0	N	N
48.M, 11y, SS,8.4, 98%	**Sz** *Sz, H, L*	?epilepsy + pain crisis		0	0	N	N	0	0	N	N
49.F,11y, SS, no Hb, 95%	**H** *H, L*	Headaches		0	0	N	N	0	0	N	N
50.M, 24y, SS, 9.9, 97.8%	**H** *H*	Headaches	HU	4 ?win-dow	0	N	N	3 ?win-dow	0	N	N
51.M, 5y, SS, no Hb, 93.5%	**H** *H*	Severe headaches		0	1 (PCA)	P (DWM)	F**P**T** O**	0	2	N	F** P** T** O**
52.M,15y, SS, 9.5, no SpO_2	**H** *H, L*	Severe headaches + chest crisis, previous MCA veloc >200 cm /sec	Tx stopped	0	1	N	F**T** O**	3	1	N	T** O**
53.M, 22y, SS, no Hb, 96%	**H, L** *H, L*	Headaches + L		0	0	N	N	0	0	N	N
54.F, 16y, SS, no Hb, 93.5%	**H, L** *H, L*	Headaches + L		4 ?win-dow	0	N	N	4 ?win-dow	0	N	N
55.M, 8y, SS, 9.9, 94.9%	**H** *H, L*	Severe headaches + previous MCA veloc >200 cm/sec	Tx	0	1	N	F**P** (SC)	0	2	N	F** P** (SC)
56.M, 22y, SS, no Hb, 92.9%	**H** *A*	Headaches		0	0	N	N	4	0	N	N

No, Sex, Age, Diagnosis, Haemoglobin (g/dl), SpO_2	Problem	Main Symptoms at Onset	Rx	R TCD	R MRA	R MRI	R Perf.	L TCD	L MRA	L MRI	L Perf.
57.M, 11y, SS, 9.1, 96.5%	**H, L** *H, L*	Headaches + L		0	1	N	N	0	1	N	N
58.F, 23y, SS, 6.9, 88%	**H** *H, L*	Headaches		0	0	FPT (DWM)	F*P*T** O**	3	1	FPTO (DWM)	F*P* T** O**
59.M, 8y, SS, no Hb, 95.5%	**H** *H*	Headaches		0	0	N	N	0	0	N	N
60.F, 9y, SS, 11.3, 97.8%	**H, L** *H, L*	Headaches + L		3	0	N	N	3	0	N	N
61.F, 14y, SS, 8.4, 94.3%	**H** *H*	Severe headaches + chest crisis	Tx stopped HU	0	1	N	N	0	1 (PCA)	N	N
62.F, 11y, SS, 9, 96.7%	**H, L** *H, L*	Severe headaches + L		3	0	N	N	3	0	N	N
63.M, 21y, SS, no Hb, 96%	**H** *H*	Headaches		0	0	N	N	3	0	N	N
64.M, 11y, SS, no Hb, 90.5%	**L** *L*	Learning difficulty, priapism, asthma	Acute Tx Broncho-dilators	0	0	N	F**P** (SC)	0	0	N	F** P** (SC)
65.M, 3y, SS, no Hb, no SpO_2	**L** *L*	Learning difficulty, severe OSA	Tonsillec -tomy CPAP	No data	2	N	Cerebellum	No data	0	N	T** O** cereb.
66.F, 11y, SS, 7.1, no SpO_2	**L** *L*	Learning difficulty		0	0	N	N	0	0	N	N
67.M, 12y, SS, 10.7, 98%	**L** *L*	Learning difficulty		0	0	N	N	0	0	N	N
68.F, 7y, SS, no Hb, 91%	**A** *A*	None		0	2	N	N	0	3	N	N
69.F, 10y, SS, no Hb, no SpO_2	**A** *L*	No neurological, recurrent chest syndrome	BMT	3	1	N	F**P**O**	0	1	N	P** O**
70.F, 7y, SS, no Hb, 98%	**A** *A*	None		0	1	F (DWM)	N	3	1	F (DWM)	N

Table 2. Cross-sectional data of 70 patients with sickle cell disease who underwent transcranial Doppler ultrasound (TCD), MRI, MRA and perfusion MRI studies.

Column 1: Hb= haemoglobin level (there is no Hb level for the patients whose haematological data is later than a year from the perfusion MRI study) ; SpO_2= day-oxygen saturation measured by pulse oximetry. *Column 2*: neurological manifestations at onset and recurrent neurological symptoms (C=coma; S=stroke; PLKE=posterior leukoencephalopathy; R=reversible ischaemic neurological deficit; T=TIA [transient ischaemic attack]; AT= anterior territory TIA; PA= posterior territory TIA; Sz=seizures; H=headaches; L= learning difficulty (cognitive); A=asymptomatic.

Column 3: hemi=hemiparesis; hemianop= hemianopia; R=right; L=left.

Column 4: Rx=treatment; Tx=transfusion; LRV= left-sided revascularisation; HU=hydroxyurea; SxD= Surgical decompression; BMT= bone marrow transplant; AED= antiepilectic drugs; ATB=antibiotics; CPAP= continuous positive airway pressure.

Columns 5 (R) and 9 (L): results of the TCD analysis (0= normal; 1= mean maximum MCA velocities > 170 < 200 cm/sec; 2= mean maximum MCA velocities $\geq$ 200 cm/sec; 3= mean maximum MCA velocities < 70 cm/sec and lowest:highest ipsilateral velocity ratio $\leq$ 0.5 or a ACA:MCA ipsilateral ratio > 1.2; 4= undetectable MCA).

Columns 6 (R) and 10 (L): worst grade of turbulence in any basal vessel on MRA (0=normal; 1 = mild turbulence; 2= moderate turbulence; 3= severe turbulence; 4= occlusion; 5= occlusion + moyamoya collaterals [MM]; PCA= posterior cerebral artery [appears when the vessel with the greatest grade of turbulence is not related to the vascular territory of abnormal MRI or perfusion MRI)]).

Columns 7 (R) and 11 (L) have data of T2-weighted MRI; and *columns 8 (R) and 12 (L)* have data of perfusion MRI: N= normal; Perf.= MRI perfusion (DSC-MRI); F=frontal; P=parietal; T=temporal; O=occipital; BG= basal ganglia; DWM= deep white matter watershed infarcts; BDZ= borderzone infarcts; SC= subcortical; cereb.= cerebellum; *= region of perfusion abnormality beyond the T2- weighted abnormality; **=region of perfusion abnormality in a territory with no T2- weighted abnormality (table adapted from Kirkham et al 2001).

Tables 3 and 4: Chapter 5.

Tables 3 and 4. Association of perfusion abnormality with progression of cerebrovascular disease and central nervous system events in sickle cell disease: longitudinal study (Chapter 5)

Table 3. Association of perfusion abnormality with TCD, MRI and MRA (longitudinal study)

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
1.M, HbSS BTx	**Coma, stroke** *PLKE, Headaches* BTx	Coma, stroke	Initial: 30/11/1999 (9 y)	No data	Normal	Multiple are of ischaemia in anterior & posterior watershed territories	R. Parietal Cortical R. parietal subcortical R. parietal/ occipital Bdz beyond infarcts	No data	Normal	Multiple are of ischaemia in anterior & posterior watershed territories	L. parietal subcortical, beyond infarcts
			Final: 11/07/2002 (12 y) **Changed Perfusion**	Normal R. MCA veloc. No US signal from L.ACA	Normal	Unchanged	R.P. C. *R. P.(SC), R. P/O Bdz, more extended* Worse (CBF)	L.MCA veloc ratio L:H <0.5	Normal	Unchanged, ***new L. caudate infarct*** **Worse**	*L.P SC., more extended* Worse (CBF)
2. M, HbSS BTx	**Coma, stroke** *Headaches, learning difficulties*	Coma, bilateral stroke w/ ICP (needed surgical de-compresion)	Initial: 25/08/1999 (13 y)	No data	R.Terminal ICA, MCA & ACA turbulence (2)	Areas of extensive cystic encephalo-malasia in R. hemisphere	R. frontal (C/SC), R. parietal (C/SC),R. temporal (C/SC) R. temp/Occip Bdz(C/SC). Beyond infarct	No data	L.Terminal ICA, MCA & ACA turbulence (2)	L .ACA territory infarct	No abnormality

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
2. M, HbSS (cont)			Final: 15/08/2001 (16 y) **Changed Perfusion**	Undectecta-ble R MCA (?window)	Unchanged	Unchanged	Beyond infarct. R. F-P-T (C/SC), R. P (C/SC) *less extended. Normal R. T/O Bdz C/SC* Better (CBF & CBV)	Undectecta-ble L. MCA (?window)	Unchanged	Unchanged	No abnormality
3. F, HbSS BTx stopped	**Coma, posterior TIA** *Headaches*	Coma, posterior TIA, seizures	Initial: 24/03/1999 (18 y)	Normal	Normal	Normal	No abnormality	L. MCA L:H Ratio veloc <0.5	Normal	Normal	No abnormality
			Final: 19/06/2001 (19 y) **Unchanged Perfusion**	Normal	Normal	Normal	No abnormality	Unchanged	Normal	Normal	No abnormality

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
4. F, HbSS BTx.	**Stroke** *Seizures, Learning difficulties*	Right hemiparesis and right focal seizures	Initial: 12/09/2002 (6 y)	Normal R MCA veloc	Normal	Ischaemic lesion in peritrigonal areas	R. Posterior Parietal (C*)* R. Superior F-P-T (SC) beyond P-T infarct	L. MCA L:H ratio veloc <0.5	Unsuccessful study, previous MRA (2001)LMC A (3). ?Now L. MCA (4)	Unsuccessful study. Previous MRI (2001): L.Precentral cortex infarct, ischaemic lesion in peritrigonal areas. ? New cerebral atrophy	L.eft P-F-T (C/SC) L T/O Bdz C/SC, beyond P-T infarct
			Final: 12/06/2003 (7 y) **Changed Perfusion**	Normal R.MCA	Normal	Unchanged	***R. Post P. C*** R. Sup F/P/T SC **Better** (MTT)	L. MCA L:H ratio veloc <0.5	L.MCA (4)	Unchanged lesions, *?progression of cortical atrophy in L MCA territory* **Worse**	L.P-F-T. F/P/T (C/SC), *L T/O Bdz C/SC, less extended* **Better** (MTT & CBF)
5. F, Hb SS BTx	**Stroke** *Headaches, Learning difficulty*	R hemiparesis + Headaches	Initial: 18/12/1998 (12 y)	No data	R. A1 (1)	Normal	No	No data	L.M1 (4), A1(3), P1(1), PCA collat MM	L. F-P-BG (caudate n.)	L Frontal (C/SC), Temporal (C), F/T Bdz (C/SC), *beyond infarct.*

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
5. F, Hb SS **(cont)**			Final: 12/10/2001 (16 y) **Changed Perfusion**	R MCA Low:High Ratio Veloc <70 cm/sec	R. A1 (1)	Normal	No	L MCA L:H V< 70 cm/s	L. M1 (4) ***A1 (3→4)*** P1 (1), P2 (1), MM **Worse**	L. F-P-BG (caudate n.) No new inf.	***L Frontal (C/ SC)*** ***Temporal (C)*** ***L F/T Bdz (C/SC)***, beyond infarct. **Worse MTT & CBF** (Improved CBV)
6.F, HbSS BTx	**Stroke** *Anterior TIA, seizures, Headaches, learning difficulties*	R hemi+ Sz+ Aphasia	Initial: 29/06/1999 (12 y)	R. MCA L:H vel <0.5	R. A1 (1)	R.lentiform nucleus	No	L. MCA L:H vel <0.5	TICA (2), M1 (2), A1 (2)	Large F-P-T infarct	L.Frontal (C/SC); Parietal (C)
			Final: 17/06/2002 (16 y) **Changed Perfusion**	R. MCA L:H vel <0.5	R. A1 (1)	R.lentiform nucleus	***R. Frontal (C)*** **Worse (MTT,CBF, CBV)**	L. MCA L:H vel <0.5	M1 (2), ***M2 (1)*** ***A2 (4)*** **Worse**	Large F-P-T infarct. No new infarct. ***F-P-T atrophy*** **Worse**	***L. Frontal (C/SC);*** Parietal (C) L Temporal (C/SC), beyond infarct. **Worse (MTT,CBF, CBV)**

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
7. F, HbSS BTx	**Stroke** *Anterior TIA, Headaches, Learning Difficulties*	Left hemip.	Initial: 06/12/2001 (7 y)	Undectecta-ble R. MCA/ ACA	tICA (3), M1 (3), M2 (1), A1 (3)	F-P watershed multiple infarcts, infarct in head of caudate, mild hemisp. atrophy	R. Frontal-Parietal [C] R. Parietal [C/SC] R.F/P Borderzone [SC] R.Temporal [C/SC], beyond infarcts	Normal L. MCA, undetecta-ble L. ACA.	A1 (2)	Two frontal deep watershed infarcts, no atrophy	No
			Final: 06/03/2003 (8 y) **Changed Perfusion**	Undectecta-ble R. MCA/ACA	***tICA (2→1),*** M1 (3), M2 (1), ***A1 (3→2), A2(1), P2 (1)*** **Better**	Unchanged MRI	***Normalised F-P BdZ*** Unchanged other regions but less extended. **Better (MTT & CBF**	***L. MCA L:H ratio <0.5, undectecta-ble ACA*** **Worse**	A1 (2), ***M1 (1), P1 (1)*** **Worse**	Unchanged MRI	No
8. F,SS Stopped Blood tx (autoantib)	**Stroke** *Stroke, TIAs, Headaches, Learning dif*	Right & left hemiparesis Dysphasia	Initial: 18/06/1999 (21 y)	No data	M1 (4), A1 (3), TICA & P1 (2), P2 (1), MM	F-P-T-O, multiple AWS/DWS/ PWS Slight hemisph. Atrophy	R. Parietal [C/SC] R. Temporal [SC], beyond infarct	No data	TICA, M1,A1 &P1 (2), P2 (1)	FPT(large MCA infarct), AWS/PWS/ DWS, severe hemisph atrophy	L. Temporal [SC] ?

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
8. F,SS (cont)			Final: 15/11/01 (24 y) **Changed Perfusion**	Undectecta-ble R. MCA/ ACA	M1 (4), ***M2 (4), TICA (2→4), A1 (3→2),*** MM **Worse & Better**	FPTO, multiple AWS/DWS/ PWS , ***BG (new?), generalis. Atrophy*** **Worse**	R. Parietal [C/SC] R. Temporal [SC], beyond infarct, ***new Temporal [C]*** **Worse MTT, CBF, CBV**	Undectecta-ble L. MCA, Mild increased velocity in L. ACA	***TICA(2→3),*** ***M1 (2→1),*** A1 (2) **Worse & Better**	FPT infarct, ***BG (new), generalis. Atrophy*** **Worse**	***? Extension of perf abn.*** in T lobe of L. FPT infarct. **Worse MTT, CBF, CBV**
9. M, HbSS BTx	**Stroke** *Headaches and decreased vision acuity*	Headaches, collapse	Initial 17/09/1999 (14 y)	Normal	R.P2 (4), PCA collat	R F & P, focal atrophy	R. Parietal-occipital [C]	Normal	L. P2 (4), PCA collat	L. DWM, BG	L. Occipital [C]
			Final: 14/03/2002 (16 y) **Changed Perfusion**	***Undectecta-ble R. MCA*** **Worse**	R.P2 (4), ***P1 (2),*** PCA collat **Worse**	Unchanged	Unchanged	***L.MCA L:H ratio <0.5*** **Worse**	L. P2 (4), ***P1 (2)*** PCA collat **Worse**	L. DWM, BG ***?occipital atrophy*** **Worse**	***L. Occipital [C]*** **Worse (MTT)**

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
10. M, HbSS BTx-Stopped BMT	**Stroke** *Anterior TIA*	Aphasia and right hemip.	Initial: 02/07/1999 (10 y)	R. MCA veloc, L:H ratio < 0.5	TICA (3), M1 (3), moyamoya	Deep watershed frontal-parietal infarcts (two infarcts)	R. Parietal [C/SC], R.P/O Bdz C/SC, R.Temporal [C], R. T/O Bdz [C/SC], beyond inf.	L. MCA veloc, L:H ratio < 0.5	TICA (3), M1 (3), moyamoya	Infarct F-P	L.Parietal [C], L.P [SC], L. P/Occip. [C] L.T/O Bdz [C], beyond infarct
			Final: 20/12/2002 (13 y) **Changed Perfusion**	***Undectecta-ble R MCA*** **Worse**	TICA (3), M1 (3), moyamoya, ***more collaterals*** **Worse**	Unchanged	***R. P[C/SC],*** ***R.P/O Bdz C/SC,*** ***R.T [C],*** ***R.T/O Bdz [C/SC],*** >> extended **Worse MTT, CBF, CBV** **Better CBV**	***Undectecta-ble L.MCA*** **Worse**	TICA (3), M1 (3), moyamoya, ***more collaterals*** **Worse**	Unchanged	L.P [C]. ***L.P [SC]*** L. P/O [C.] ***L.T/O Bdz [C], more extended*** **Worse MTT, CBF, CBV**
11. M. HbSS BTx	**Anterior TIA** *Asymptoma-tic*	TIAs	Initial: 13/12/2001 (15 y)	R. MCA veloc L:H ratio < 0.5	M2 (1)	No	No	R. MCA veloc L:H ratio < 0.5	No	Small F-P subcortical white matter infarcts	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
11. M. HbSS (cont.) BTx			Final: 25/07/2003 (16 y) **Unchanged Perfusion**	R. MCA veloc L:H ratio < 0.5	Unchanged	No	R. Sup. Parietal [C], different slice orientation to compare w/previous study	L. MCA veloc L:H ratio < 0.5	Unchanged	Unchanged	No
12. F, HbSS BTx-stopped	**Anterior TIA** *Anterior TIA, Headaches, Learning &, behaviour problems*	Transient hemiparesis, headaches	Initial: 23/11/1999 (21 y)	R. MCA veloc L:H ratio < 0.5	M1(2)	Small anterior deep watershed infarctsn and basal ganglia (F-T)	R Frontal C/SC R. Parietal-Temporal [SC (WM)], beyond infarct	L MCA veloc L:H ratio < 0.5	TICA (3), M1 (3), M2 (2), A1 (3)	Small deep white matter infarct and basal ganglia (T)	L. Parietal [C], L. Parietal-Temporal [SC (WM)], beyond infarct
			Final: 07/12/2001 (25 y) **Unchanged Perfusion**	***Undectectable R. MCA (thick skull)***	***M1 (2), A1 (2)*** **Worse**	Unchanged	Unchanged	***Undectectable L. MCA (thick skull)***	***TICA (3→0), M1 (3→1), M2 (2→1), A1 (3→0)*** **Better**	Unchanged	Unchanged

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
13. F, HbSS BTx, AED (Carbama-zepine)	**Anterior TIA** *Anterior TIA, Seizures, Headaches*	Transient L & R. hemiparesis, Focal seizures, Headaches	Initial: 30/11/1999 (8 y)	R. MCA veloc >200 cm/s	R. M1 (3), M2 (1), A1 (2)	R.T/DWS (corona radiata)	R. Frontal-Parietal [C/SC]; R. Temporal [C] RT/O Bdz C/SC R.T. SC, beyond infarct	L MCA normal veloc,	L. A1 (3), M1 (1)	No	No
			Final: 04/01/2002 (11 y) **Changed Perfusion**	R. MCA veloc >200 cm/s ***And R MCA vel. L:H ratio <0.5 at 4 cm depth*** **Worse**	R. ***M1 (3→4), M2 (1→2), A1 (2→0), PCA collat*** **Worse & Better**	R.T/DWS (corona radiata) Unchanged, no new infarct	***R. F-P [C/SC]*** ***R. T.[C]*** RT/O Bdz [C/SC] R.T. [SC] Beyond infarct, *new areas of perfusion abnormality* **Worse (MTT & CBF)** **Better CBV**	***L MCA veloc L:H <0.5*** **Worse**	L. ***A1 (3→0),*** M1 (1) **Better**	No	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
14. **M, HbSS** BTx-stopped Left indirect Frontal revasculari-sation Hydroxyurea	**Anterior TIA** *Anterior TIA, Headaches, Learning Difficulties*	TIA (transient right hemip.), Headaches	Initial: 18/06/1999 (12 y)	Undectecta-ble R. MCA	A1 (2)	Multiple small ACA/MCA watershed infarcts, mild parietal lobe atrophy, mild global atrophy	R. P C R. Parietal [SC (WM)] R.P/O Bdz [SC] R. T/O Bdz [C/SC] R.O [C]; beyond infarct.	Normal MCA	M1 (1), M2 (2), A1 (1)	Multiple small ACA/MCA watershed infarcts, and small infarct in head of caudate nucleus. Mild parietal lobe atrophy, mild global atrophy	L. P [SC] (WM) L. P [C/SC] L. P/O Bdz L. T [SC (WM)] L.T/O Bdz [C/SC] L. O [C], beyond infarct
			Final: 27/08/2002 (15 y) **Changed Perfusion-Better** Worsening only in one region MTT and CBF, and another region CBV on the left perfusion.	***Normal MCA, undectecta-ble ACA*** **Better**	***TICA (2), M1 (1)***, A1 (2) **Worse**	Unchanged	***R. P C*** R. P SC (WM) **R.P/O Bdz** R. T/O Bdz R.O C; Be-yond infarct, *less extended* **Better (MTT, CBF & CBV)**	***L. MCA veloc L:H ratio <0.5, undetectable ACA.*** **Worse**	***TICA (1). M1 (1→0), M2 (2→1),*** A1 (1) **Better & Worse**	Unchanged	L. P [SC] (WM) ***L. P [C/SC]*** ***L. P/O Bdz*** ***L. T [SC (WM)]*** L.T/O Bdz [C/SC] ***L. O [C],*** Beyond infarct, *less extended.* **Better MTT, CBF,CBV**

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
15. M, HbSS BTx-stopped Hydroxyurea	**Posterior TIA** *Headaches*	Posterior TIA-headaches	Initial: 30/07/1999 (15 y)	Normal	No	Normal	Normal	Normal	No	Normal	Normal
			Final: 04/02/2002 (19 y) **Changed Perfusion**	***R MCA veloc L:H ratio < 0.5*** **Worse**	No	Normal	***R. Temporal [C]*** **Worse (MTT, slightly CBF)**	Normal	No	Normal	Normal
16. F, HbSS BTx-stopped	**Posterior TIA** *Posterior TIA, Headaches, Learning Difficulties*	Posterior TIA (mild bilateral hemiparesis, VIth nerve paresis) and headaches	Initial: 30/07/1999 (11 y)	R MCA veloc L:H ratio < 0.5	TICA (1), M1 (1)	No	R Temporal [C]	L MCA veloc L:H ratio < 0.5	TICA (1), M1 (2), A1 (1)	No	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
16. F, HbSS (cont) BTx-stopped			Final: 12/11/2001 (13 y) **Changed Perfusion**	***Undetectable R. MCA (?window)*** **?Worse**	***TICA (1→0), M1 (1→0)*** **Better**	Three small F-P deep watershed infarcts. **Worse**	***Normalised R Temporal [C]*** **Better (MTT)**	***Undetectable L. MCA (?window)*** **?Worse**	***TICA (1→0), M1 (2→1), A1 (1→0)*** **Better**	Small multiple MCA/PCA deepwatershed infarcts. Mild MCA territory atrophy **Worse**	No
17. F, HbS/B^0 thalassaemia	**Seizures** *Headaches*	Seizures in infancy	Initial: 01/09/1999 (16 y)	R.MCA veloc L:H ratio <0.5	Normal	Normal	No	Normal	Normal	Normal	No
			Final: 03/07/2001 (17 y) **Unchanged Perfusion**	***Normal*** **Better**	Normal	Normal	No	Normal	Normal	Normal	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
18. F, HbSS Chronic hypoxemia	**Headaches** *Headaches, Learning difficulties*	**Headaches**	Initial: 17/06/1999 (8 y)	Normal	R. TICA (1), M2 (1)	Normal	No	Normal	M2 (2)	Normal	No
			Final: 08/03/2002 (11 y) **Unchanged Perfusion**	Normal	***R. TICA (1→0), M2 (1→0)*** **Better**	Normal	No	Normal	***M2 (2→0)*** **Better**	Normal	No
19. M, HbSS Hydroxyurea	**Headaches** *Headaches*	Headaches	Initial: 24/03/1999 (22 y)	No data	No	Normal	No	No data	No	Normal	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
19. M, HbSS (cont) Hydroxyurea			Final: 11/07/2001 (24 y) **Unchanged Perfusion**	Undetecta-ble R MCA (thick skull, ?window))	No	Normal	No	L MCA veloc L:H ratio <0.5 (thick skull, ?window)	No	Normal	No
20. M, HbSS BTx-stopped	**Headaches** *Headaches, Learning and Behaviour problems*	Severe headaches, chest syndrome	Initial: 24/02/1999 (12 y)	R. MCA veloc >200 cm/sec	M2 (2), P1 (2)	Normal	R. Frontal [C/SC], R.Temporal [C/SC], R.T/ Occip. Bdz [C/SC]; R.T/O Bdz [C]	L. MCA veloc L:H , 0.5	M2 (1)	Normal	L.Temp./Occ ipital Bdz; L.T-Occipital [C].
			Final: 29/08/2002 (15 y) **Changed Perfusion**	***Normalised TCD*** **Better**	***TICA (1 or 2), M2 (2→0), P1 (2→0)*** **Better & Worse**	Normal	R. F [C/SC], ***Normalised R.T [C/SC], R.T/O Bz [C/SC];*** R.T/O Bdz [C] **Better (MTT & CBF)**	L. MCA veloc L:H , 0.5	***TICA (1 or 2), M2 (1→0)*** **Better & Worse**	Normal	***L. T/O Bdz L. T-O [C].*** **Better (MTT & CBF)** Worsening of post. T region (CBV) after stopped BTx

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
21. M, HbSS	**Headaches** *Headaches, Learning Difficulties*	Headaches, learning difficulties	Initial: 03/11/1999 (20 y)	No data	No	Normal	No	No data	No	Normal	No
			Final: 22/10/2001 (22 y) **Unchanged Perfusion**	Normal	No	Normal	No	Normal	No	Normal	No
22. F, HbSS	**Headaches** *Headaches, learning difficulties*	Headaches, learning difficulties	Initial: 06/07/1999 (13 y)	R ACA:MCA ➢ 1.2	No	Normal	No	L.MCA veloc L:H ratio <0.5	No	Normal	No

Case, Sex, Dx Therapy	Main Symptom at presentation *Recurrent Symptom(s)*	Onset Symptoms	Date of Study (age)	Right TCD	Right MRA Turbulence	Right MRI Infarct	Right Perfusion Abnormal	Left TCD	Left MRA Turbulence	Left MRI Infarct	Left Perfusion Abnormal
22. F, HbSS (cont)			Final: 10/10/2001 (16 y) **Unchanged Perfusion**	***Undetectable R MCA (?window)*** **?Worse**	No	Normal	No	***Undetectable L MCA (?window)*** **?Worse**	No	Normal	No
23. M, HbSS	**Headaches** *Headaches, Learning Difficulties*	Headaches, Learning Difficulties	Initial: 30/06/1999 (8 y)	Normal	No	Normal	No	Normal	No	Normal	No
			Final: 20/07/2001 (11 y) **Unchanged Perfusion**	Normal	***R. A1 (1)*** **Worse**	Normal	No	Normal	***R. M1 (1), M2 (1)*** **Worse**	Normal	No

Table 3. Association of perfusion abnormality with transcranial Doppler ultrasound (TCD), MRI and MRA. Longitudinal study.
Dx: diagnosis; *F*: female; *M*: male, *SS* : homozygous sickle cell anaemia; *TIA*: transient ischaemic attack; *Diff:* difficulties; *BTx:* transfusion; *AED*: antiepileptic medication; *L*: left; *R*: right; *hemi*: hemiparesis; *PLKE:* posterior leukoencephalopathy; *TICA*: terminal internal carotid artery; *MCA*: middle cerebral artery; *M1 & M2*: middle cerebral artery segments 1 & 2; *A1 & A2*: anterior cerebral artery segments 1 & 2, *P1 & P2*: posterior cerebral artery segments 1 & 2; *MM*: moyamoya collaterals; *vel*: mean MCA velocity; *F*: frontal lobe; *T*: temporal lobe; *P*: parietal lobe; *O*: occipital lobe; *AWS*: anterior watershed; *PWS*: posterior watershed; *DWS*: deep watershed; *DWM*: deep white matter; *BG*: basal ganglia. *MCA L:H*: MCA lowest: highest mean ipsilateral velocity ratio; *ACA:MCA*: ACA:MCA highest ipsilateral velocities ratio.
MRA turbulence classified as: *(1):* mild; *(2)*: moderate; *(3)*: severe and *(4)*: occlusion.
MTT: Mean transit time
CBF: Cerebral Blood Flow
CBV: Cerebral Blood Volume
C: Cortical
SC: Subcortical

Worsening of perfusion MRI parameters (MTT, CBF or CBV)

Improvement (better) of perfusion MRI parameters (MTT, CBF or CBV)

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
1.HbSS	I: 30/11/1999 (9 y)	Right Hemisp.						No parameter
		R.P. C.	=	Unchanged	=	-	=	
Coma, stroke	F: 11/07/2002	***R. F. SC.***	++	Unchanged	=	- -	+	
Coma (Posterior Leukoencepha-lopathy)	(12 y)	***R. P.SC.***	++	Unchang. :>ext.	=	- -	+	
	I: SVD	***R. P/O Bdz***	=	Unchanged	=	- -	=	
	F: Sum. Param.							
	TTP similar to MTT	Left Hemisp.						
BTx	MPC similar to CBF	***L. F. SC***	++	Unchanged	=	- -	+	
		L. P SC.	++	Unchang: >ext.	=	- -	+	
	Worse (CBF)							
2. HbSS	I: 25/08/1999	Right hemisp.						
	(13 y)	R. F C/SC	+++	Unchanged	- - -	Unchanged	- - -	Unchanged
Coma, stroke	F: 15/08/2001	R. P C/SC	+++	Unchanged	- - -	Unchanged	- - -	Unchanged
Headaches	(16 y)	R. T C/SC	=	Unchanged	- -	- - -	- -	- - -
		R. T/O Bdz C/SC	=	Unchanged	- - -	=	- -	=
BTx								
	Better (CBF & CBV)	Left Hemisp	=	=	=	=	=	=
3.HbSS	I: 24/03/1999	Right Hemisp.						
Coma, posterior TIA	(18 y)	R.T. SC (BWM)	+++	+++: >extended	=	Unchanged	=	Unchanged
	F: 19/06/2001							
Headaches	(19 y)	Left Hemisp.						
BTx stopped		L.P. SC (BWM)	+++	+++: >extended	=	=/-	=	Unchanged
	Unchanged	L.T. SC (BWM)	+++	+++: >extended	=	Unchanged	=	Unchanged
	(WM affected?)							

Case, Symptom at Presentation *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
4.HbSS **Stroke** *Seizures*   BTx.

Case, Symptom at Presentation *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
7. HbSS **Stroke** *Anterior TIA* BTx	I:06/12/2001 (7 y) F: 06/03/2003 (8 y) **Better (MTT & CBF)**	Right Hemisp. R.F.C R.P. C. R. P. C/SC ***R.F/P Bdz SC*** R.T C/SC Left Hemisp.	++ +++ +++ +++ +++ =	+++ Unchanged Unchanged = Unchanged Unchanged	- - - - - - - - - - - - - =	Unchanged Unchanged Unchanged = - -/- - - Unchanged	- = = - =/- =	-/- - - -/- - = Unchanged Unchanged
8. HbSS **Stroke** *Stroke* BTx (stopped)	I: 18/06/1999 (21 y) F: 15/11/2001 (24 y) **Worse (MTT, CBF & CBV)**	L. Hemisph L. T. SC R. Hemisph R. P. C. R. P. SC. R. T. C. R. T. SC.	? (different slice orientation) ++ +++ = +++	++ +++ Unchanged ++ ++	? (different slice orientation) - - - - - - = - - -	- - Unchanged Unchanged - - - -	? (different slice orientation) - - - - - - = - - -	- = = ++ -
9.HbSS **Stroke** *Headaches* BTx	I: 17/09/1999 (14 y) F: 14/03/2002 (16 y) **Worse (MTT)**	Right Hemisp. R. P C. R. O. C. Left Hemisp. *L. O.C.*	= = =	Unchanged Unchanged ++	= - - - -	- - - - - - Unchanged	- - - - - -	Unchanged - - - Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
10. HbSS **Stroke** *Anterior TIA* BTx- Stopped BMT	I: 02/07/1999 (10 y) F: 20/12/2002 (13 y) **Worse (MTT & CBF)** Improved CBV	Right Hemisp. *R.P. C* *R. P SC* *R.P/O Bdz C/SC* R.T C. R.T SC. (BWM) *R.T/O Bdz C/SC* Left Hemisp. L.P C. *L.P SC* L. P/O C. *L.T/O Bdz C*	= = = = +++ = = = = =	+++ +++ Unchanged + Unchanged + Unchanged +++ Unchanged +++	- - - = - - - = - = - - - = = =	Unchanged - - - - - - - - - - - Unchanged - - - - - - - -	- - - - - - - - - = = = - - - = = =	Unchanged - - - - - Unchanged - - - -
11. HbSS **Anterior TIA** *Asymptomatic* BTx	I: 13/12/2001 (15 y) F: 25/07/2003 (16 y) **Unchanged**	Right Hemisp. R. Sup. P. C Left Hemisphere	? (diff. slice orientation) =	+/++ Unchanged	? (diff. slice orientation) =	- - Unchanged	? (diff. slice orientation) =	- Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
12. HbSS	I: 23/11/1999 (21 y)	Right hemisp.						
		R.F C/SC	? (orientation)	++	? (orientarion)	-	? (orientation)	=
Anterior TIA	F: 07/12/2001 (25 y)	R.P SC. (BWM)	+++	Unchanged	- - -	Unchanged	- -	Unchanged
Anterior TIA		R.T SC (BWM)	++	+++	=/-	-	=/-	-
BTx- stopped	**Unchanged**	Left hemisp.						
		L. P C	+++	? (orientation)	- - -	? (orientation)	-/=	? (orientation)
		L. P SC (BWM)	+++	Unchanged	- -/- - -	- - -	- -	Unchanged
		L.T SC (BWM)	+++	Unchanged	=/-	- -	=/-	-
13- HbSS	I: 30/11/1999 (8 y)	Right Hemisp.						
		R. F. C/SC	=	++	++	-	++	=
Anterior TIA	F: 04/01/2002 (11 y)	***R. P. C/SC***	=	++	++	- -	++	+
Anterior TIA		***R. T. C***	=	+++	+++	Unchanged	++	+++
		RT/O Bdz C/SC	++	+++	-	- -	=	+
BTx- AED	**Worse (MTT & CBF)**	R.T. SC	++	Unchanged	-	Unchanged	=	Unchanged
	Improvement CBV	L hemisp.	=	Unchanged	=	Unchanged	=	Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
14. HbSS **Anterior TIA** *Anterior TIA* BTx-stopped Left indirect frontal revascularisation Hydroxyurea	I: 18/06/1999 (12 y) F: 27/08/2002 (15 y) **Better (MTT, CBF & CBV)** Worsening only in one region MTT and CBF, and another region CBV.	Right Hemisp ***R. P C*** R. P SC (BWM) R. P C/SC ***R.P/O Bdz*** R. T/O Bdz R.O C Left Hemisp L. P C L. P SC (BWM) ***L. P C/SC*** ***L. P/O Bdz*** L. T SC (WM) L.T/O Bdz C/SC ***L. O C***	= +++ = ++ + + Diff. orientation +++ = ++ +++ ++ ++	Unchanged Unchanged Unchanged = = = +++ ++ +++ = ++ ++ =	- - = = - - - Diff.orientation = =/- - -/= - - - -	= Unchanged Unchanged = = = Unchanged =/- - - = = Unchanged =	-/- - = = = - - Diff. orientation = = = = - - - -	= Unchanged Unchanged Unchanged = = =/+ =/- =/+ Unchanged - - Unchanged =
15. HbSS **Posterior TIA** *Headaches* BTx- stopped Hydroxyurea	I: 30/07/1999 (15 y) F: 04/02/2002 (19 y) **Worse (MTT, slightly CBF)**	Right Hemisp. ***R.T. C*** L. hemisphere	= =	+++ Unchanged	= =	- Unchanged	= =	Unchanged Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
16. HbSS **Posterior TIA** *Posterior TIA* BTx- stopped	I: 30/07/1999 (11 y) F: 12/11/2001 (13 y) **Better (MTT)**	Right Hemisp. *R. T. C* Left Hemisp.	++ =	= Unchanged	=/- =	= Unchanged	- =	= Unchanged
17. HbSS **Seizures** *Headaches*	I: 01/09/1999 (16 y) F: 03/07/2001 (17 y) I: SVD F: Sum. Param. TTP similar to MTT MPC similar to CBF **Unchanged**	Right Hemisp Left Hemisp	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Not done (Sum. Param)
18.HbSS **Headaches** *Headaches*	I: 17/06/1999 (8 y) F: 08/03/2002 (11 y) **Unchanged**	Right Hemisp. Left Hemisp.	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Unchanged Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
19. HbSS **Headaches** *Headaches* Hydroxyurea	I: 24/03/1999 (22 y) F: 11/07/2001 (24 y) I: SVD F: Sum. Param. TTP similar to MTT MPC similar to CBF **Unchanged**	Right Hemisp. Left Hemisp.	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Not done (SumParameters)
20. HbSS **Headaches** *Headaches* BTx-stopped	I: 24/02/1999 (12 y) F: 29/08/2002 (15 y) **Better (MTT & CBF)** Worsening of post. T region (CBV) after stopped BTx	Right Hemisp. R. F C/SC ***R.T C/SC*** ***R.T/O Bz C/SC*** R.T/O Bdz C Left Hemisp **L. T/O Bdz** ***L.O C.***	+++ +++ +++ +++ +++ ++	++ = = Unchanged ++ +++	- - - - - - - - - - - - - - - - -	-/- - = = - -/- - - - - -/- - -	- - - - - =	- - = = - - = - -
21. HbSS **Headaches** *Headaches*	I: 03/11/1999 (20 y) F: 22/10/2001 (22 y) **Unchanged** Prominent deep WM	Right Hemisp. Left Hemisp.	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Unchanged Unchanged

Case, Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
22. HbSS **Headaches** *Headaches*	I: 06/07/1999 (13 y) F: 10/10/2001 (16 y) **Unchanged**	Right Hemisp. Left Hemisp.	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Unchanged Unchanged
23.HbSS **Headaches** *Headaches*	I: 30/06/1999 (8 y) F: 20/07/2001 (11 y) I: SVD F: Sum. Param. TTP similar to MTT MPC similar to CBF **Unchanged**	Right Hemisp. Left Hemisp.	= =	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Not done (Sum. Param)

Table 4. Longitudinal changes of perfusion MRI parameters (mean transit time [MTT], cerebral blood flow [CBF] and cerebral blood volume [CBV]) in sickle cell patients through time.

Column 1: *HbSS*= homozygous sickle cell disease (sickle cell anaemia); *TIA*= transient ischaemic attack; *BTx*= blood transfusion; *BMT*= bone marrow transplant; *AED*= antiepileptic drugs.

Column 3: *hemisph.*= cerebral hemisphere, *F*= frontal region; *P*= parietal region; *T*= temporal region; *O*= occipital region; *C*= cortical; *SC*= subcortical; *BWM*= bright white matter (?abnormal) and *Bdz*= borderzone.

Column 2,4,5,6,7,8 and 9: The table compared the initial (first perfusion MRI scan) and final (last perfusion MRI scan) changes of every parameter. *DSC-MRI*= dynamic susceptibility contrast MRI (perfusion MRI); *MTT*= mean transit time; *CBF*= cerebral blood flow; *CBV*= cerebral blood volume; *SVD*= singular value deconvolution (to create MTT,CBF and CBV maps); *sum. param:* summary Parameters; *TTP*= time-to-peak; *MPC*= mean peak concentration; *unchang.*= unchanged; *ext.*= extended; and *diff*= different.
MTT and *CBF* were the main parameters to assess the grade of change in cerebral perfusion (better, unchanged or worse perfusion MRI) of every patient longitudinally. Perfusion MRI parameters are assessed following a scale of worsening cerebral blood flow:
=: equal or normal
- (for CBF and CBV): decreased passage of IV Gadolinium (-: mild, --:moderate, ---: severe)
+(for MTT): increased the mean transit time of the passage of IV Gd (+: mild, ++: moderate, +++ severe)
+ (for CBF/CBV): increased the passage of IV Gd (increased blow flow; +: mild, ++: moderate, +++: severe)
Based on the equation: *MTT= CBV/ CBF*. A significant change of the perfusion MRI parameters was defined in the improvement or worsening of the parameter (MTT, CBF, CBV) in two grades of the scale (i.e. from '= to - -'; or '+++ to +'), to avoid bias of perfusion graduation in the visual assessment of the perfusion maps of MTT, CBF and CBV by different examiners.

Worsening of perfusion MRI parameters (MTT, CBF, CBV)

Improvement (better) of perfusion MRI parameters (MTT, CBF, CBV)

Tables 5 and 6. Effect of Blood transfusion therapy on perfusion abnormality in the short-term.

Case (No. table 3), Sex, Age, Dx, Hb (g/dL)	Problem, Therapy, BTx Date	Onset Symptoms	Date of Study (SpO_2 %; BP mmHg)	Right TCD	Right MRA Turbulence	Right MRI	Right Abnormal Perfusion	Left TCD	Left MRA Turbulence	Left MRI	Left Abnormal Perfusion
1. (11), M, 15 y, SS, no Hb	Anterior TIAs Blood Tx	TIA, intracranial aneurysms	**Pre-BTx 13/12/2001** (97; 115/49)	R. MCA vel <70 cm/sec & L:H ratio <0.5	Normal	Normal	Normal	L. MCA vel <70 cm/sec & L:H ratio <0.5	Normal	F-P (DWM)	Normal
	Blood Tx 15/12/2001		**Post-BTx: 20/12/2001** (97; 121/67)	Unchanged	***M2 (0→1)*** **Worse**	Normal	Unchanged	Unchanged	Normal	Unchanged	Unchanged
2. (5), F, 16 y, SS, no Hb	Stroke, H, L. Blood Tx	R hemi + H	**Pre-BTx 12/10/2001** (98; 108/48)	R MCA vel< 70 cm/sec & L:H ratio <0.5	R. A1 (1)	Normal	Normal	L MCA vel < 70 cm/s	L. M1 (4) A1 (4) P1 (1), P2 (1), MM	L. F-P-BG (caudate nucleus)	L F (C/ SC) T (C); L F-T Bdz (C/SC), beyond infarct.
	Blood Tx 19/10/2001		**Post-BTx 25/10/2001** (99; 108/55)	***Normal*** **Improved**	Unchanged	Normal	Normal	Unchanged	L. M1 (4) A1 (4) ***P1 (1→4), P2 (1→4),*** MM **Worse**	Unchanged	***L. frontal pole (C),*** unchanged rest. **Improved MTT**
3. (2), M, 16 y, SS, 9.2	Stroke, coma, H, L. Blood Tx	Bilateral Stroke with coma, increased ICP	**Pre-BTx 15/08/2001**	Undetectable MCA	TICA (2), MCA (2), ACA (2)	Extensive cystic encephalomalacia in the ACA/MCA territories	R.Temporal (C/SC)	Undetectable MCA	TICA (2), MCA (2), ACA (2)	L. ACA territory infarct	Normal
	Blood Tx 22/06/2001		**Post-BTx (04/07/2001)**	Unchanged	Unchanged	Unchanged	***R. Temporal (C/SC)*** **Improved CBF & CBV**	Unchanged	Unchanged	Unchanged	Unchanged

Case (No. table 3), Sex, Age, Dx, Hb (g/dL)	Problem, Therapy, BTx Date	Onset Symptoms	Date of Study (SpO_2 %, BP mmHg)	Right TCD	Right MRA Turbulence	Right MRI	Right Abnormal Perfusion	Left TCD	Left MRA Turbulence	Left MRI	Left Abnormal Perfusion
4. (6), F, 16 y, SS, no Hb	Stroke Blood Tx	R hemi + Sz + Aphasia	**Pre-BTx** **17/06/2002** (98; 112/75)	MCA L:H <0.5	R. A1 (1)	R. T (BG)	R. Frontal (C)	L MCA vel < 70 cm/s	M1 (2), M2 (1) A1 (4)	Large F-P-T infarct. FPT atrophy	L. F (C/SC); L. P (C) L T (C/SC), beyond infarct
	Blood Tx **20/06/2002**		**Post-BTx** **24/06/2002** (98; 115/73)	Unchanged	Unchanged	Unchanged	***R. frontal pole (C).*** **Improved CBV**	Unchanged	Unchanged	Unchanged	***L. frontal pole (C)*** **Improved CBV**
5. M, 16y SS, no Hb	Posterior TIA, H Blood Tx	Collapse + transient loss of vision	**Pre-BTx** **24/03/2003** (92; 85/60)	MCA vel < 70 cm/sec	Normal	Frontal DWS small infarcts in ACA/MCA territory	R. Frontal C/SC	Normal	A (1)	Frontal DWS small infarcts in ACA/MCA territory	Left Frontal (C/SC)
	Blood Tx **26/03/2003**		**Post-BTx** **27/03/2003** (94; 102/54)	***Normal*** **Improved**	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	***Left Frontal C/SC*** **Improved MTT/CBF**
6. F, 27 y, SS, 9.3	Stroke Blood Tx	Sz, collapse	**Pre-BTx** **12/03/2002** (96; 105/57)	Normal MCA	TICA (3), M1 (2), M2 (1), A1 (2), P1 (1)	R. P infarct Cerebral atrophy	R. superior P-O (C), R .inferior P-O (C) beyond infarct. R. T (SC).	Normal MCA, decreased L. PCA velocity	P1 (1)	P infarct watershed (MCA/PCA) Cerebral atrophy	L.superior P-O (C) limited to infarct area
	Blood Tx **18/03/2002** **Blood Tx** **12/04/2002** (Perfusion)		**Post-BTx** **22/03/2002** (MRI-MRA-TCD) **12/04/2002** (Perfusion) (97; 110/52)	Unchanged	TICA (3), ***M1 (2→1), M2 (1→0), A1 (2→0), P1 (1→0), P2 (1)*** **Improved**	Unchanged	***R. superior P-O (C). Unchanged other regions*** **Improved MTT**	Unchanged	***P1 (1→0), P2 (1)***	Unchanged	***L. superior P-O (C).*** **Improved CBF / CBV**

Case (No. table 3), Sex, Age, Dx; Hb (g/dL)	Problem, Therapy, BTx Date	Onset Symptoms	Date of Study (SpO_2 %, BP mmHg)	Right TCD	Right MRA Turbulence	Right MRI	Right Abnormal Perfusion	Left TCD	Left MRA Turbulence	Left MRI	Left Abnormal Perfusion
7. (7), F, 8y, SS, no Hb	Stroke, anterior TIAs, H, L Blood Tx	Left hemi	**Pre-BTx 06/03/2003** (no SpO_2/ BP)	R. MCA vel.< 70 cm/sec	TICA (2), M1 (3), M2 (1), A1 (2), A2 (1), P2 (1)	Large MCA/ACA (DWS) infarct + head of caudate infarct	R. superior F-P (C), R. inferior F-P-O (C/SC), R. T (C/SC)	L. MCA vel < 70 cm/sec.	M1 (1), A1 (2), P1 (1)	Frontal DWS infarct	L.T (SC)
	Blood Tx 08/03/2003		**Post-BTx 13/03/2003** (no SpO_2, 104/49)	Unchanged	TICA (2), M1 (3), ***M2 (1→3), A1 (2→3), A2 (1→2),*** P2 (1) **Worse**	Unchanged	Unchanged	Unchanged	***M1 (1→0),*** A1 (2), ***P1 (1→0)*** **Improved**	Unchanged	Unchanged
8. (9), M, 17 y, SS, 10.1	Stroke, H, decreased visual acuity. Blood Tx	H, collapse	**Pre-BTx 14/03/2002** (97; 121/31)	Undetectable MCA. Decreased R. PCA velocity	P1 (2), P2 (4), PCA collat	R. F - P infarct, focal atrophy	R. P-O [C]	L.MCA L:H ratio <0.5	L. P2 (4), P1 (2) PCA collaterals	L. DWM, BG ?occipital atrophy	L. O [C]
	Blood Tx 16/03/2002		**Post-BTx 21/03/2002** (99; 119/46)	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged

Table 5. Association of perfusion abnormality with transcranial Doppler ultrasound (TCD), MRI and MRA : Pre- and post-blood transfusion study.

Dx: diagnosis; *F*: female; *M*: male, *SS*: homozygous sickle cell anaemia; *S*: stroke; *TIA*: transient ischaemic attack; *H:* headaches; *L*: learning difficulties; *Sz*: seizures; *BTx:* blood transfusion; *Hb:* haemoglobin; *L*: left; *R*: right; *hemi*: hemiparesis; *TICA*: terminal internal carotid artery; *MCA*: middle cerebral artery; *M1 & M2*: middle cerebral artery segments 1 & 2; *A1 & A2*: anterior cerebral artery segments 1 & 2, *P1 & P2*: posterior cerebral artery segments 1 & 2; *MM*: moyamoya collaterals; *vel*: mean MCA velocity; *F*: frontal lobe; *T*: temporal lobe ; *P*: parietal lobe; *O*: occipital lobe; *AWS*: anterior watershed; *DWS*: deep watershed; *DWM*: deep white matter; *BG*: basal ganglia. *MCA L:H*: MCA lowest: highest mean ipsilateral velocity ratio.
MRA turbulence classified as: *(1):* mild; *(2)*: moderate; *(3)*: severe and *(4)*: occlusion.
MTT: Mean transit time; *CBF*: Cerebral Blood Flow; *CBV*: Cerebral Blood Volume; *C*: Cortical; *SC*: Subcortical.

Worsening of perfusion MRI parameters (MTT, CBF or CBV)

Improvement (better) of perfusion MRI parameters (MTT, CBF or CBV)

Case, (No. table 3), Sex, Age, Dx, Hb (g/dL), Problem, BTx Date	MRI –DSC Date	Perfusion Abnormality	MTT- Pre-BTx	MTT-Post-BTx	CBF-Pre-BTx	CBF-Post-BTx	CBV- Pre-BTx	CBV-Post-BTx
1. (11), M, 15 y, SS, no Hb Anterior TIAs Blood Tx 15/12/2001	Pre-BTx 13/12/2001 Post-BTx 20/12/2001	*Right Hemisp.* R.P. SC (BWM) *Left Hemisp.* L.P.SC (BWM)	+++ +++	Unchanged Unchanged	= =	Unchanged Unchanged	= =	Unchanged Unchanged
2. (5), F, 16 y, SS, no Hb Stroke, H, L. Blood Tx 19/10/2001	Pre-BTx 12/10/2001 Post-BTx 25/10/2001	*Right Hemisp.* *Left Hemisp.* ***L.F pole*** L.F C L.Inferior F.C L.P C/SC L.T C/SC L.inferior T C L inf T SC (BWM) L. T/O C Bdz	= +++ +++ +++ +++ +++ = +++ ++	= = Unchanged Unchanged Unchanged Unchanged ++ ++ +++	= - - - - - - - - - - - - - - = =/- - -	= Unchanged Unchanged - - - Unchanged - - - - Unchanged	= =/- =/- =/+ - - - = = = =	= Unchanged - - Unchanged - - Unchanged Unchanged Unchanged Unchanged
3. (2), M, 16 y, SS, 9.2 Stroke, coma, H, L Blood Tx 22/06/2001	Pre-BTx 15/08/2001 Post-BTx 04/07/2001	*Right Hemisp.* ***R. T. C/SC*** R.inf T C/SC *Left Hemisp.*	= = =	Unchanged Unchanged =	- - - - - - =	- - - =	- - - - - - =	- -/- =

Case, (No. table 3), Sex, Age, Dx, Hb (g/dL), Problem, BTx Date	MRI –DSC Date	Perfusion Abnormality	MTT- Pre-BTx	MTT-Post-BTx	CBF-Pre-BTx	CBF-Post-BTx	CBV- Pre-BTx	CBV-Post-BTx
4. (6), F, 16 y, SS, no Hb Stroke Blood Tx 20/06/2002	Pre-BTx 17/06/2002 Post-BTx 24/06/2002	*Right hemisp.* ***R. F pole*** *Left hemisp.* ***L.F pole*** L.FC/SCsuperior L.FC/SCinferior L.F/T Bdz C/SC L.T C/SC	+++ +++ ++ +++ + +	++ ++ Unchanged ++ +/++ ++	- - - - - - - - - - - - -	- - Unchanged Unchanged -- -- --	- - - - - - = - - - - -	- - Unchanged - - - - - -
5. M, 16y SS, no Hb Posterior TIA, H Blood Tx 26/03/2003	Pre-BTx 24/03/2003 Post-BTx 27/03/2002	*Right hemisp.* R. F C/SC R. P SC (BWM) R.T SC (BWM) *Left hemisp.* ***L. F C/SC*** L. P SC (BWM) L. T SC (BWM)	++ +++ +++ (>extended) ++ +++(>extended) +++	Unchanged Unchanged Unchanged (less extended) = Unchanged (less Unchanged	- - = = - - = =	-/- - Unchanged Unchanged = Unchanged Unchanged	- = = - = =	Unchanged Unchanged Unchanged = Unchanged Unchanged
6. F, 27 y, SS, 9.3 Stroke Blood Tx 18/03/2002 (For Pre-BTx) Blood Tx 12/04/2002 (For Post-BTx	Pre-BTx 12/03/2002 Post-BTx 12/04/2002	*Right hemisp.* ***R. Sup P/O C*** R.Inf. P/O C *Left hemisp* ***L. Sup P/O. C.***	+++ +++ +	+ Unchanged =	- - - - - - - -	- - Unchanged =	- - - - - - - -	- - Unchanged =

Case, (No. table 3), Sex, Age, Dx, Hb (g/dL), Problem, BTx Date	MRI –DSC Date	Perfusion Abnormality	MTT- Pre-BTx	MTT-Post-BTx	CBF-Pre-BTx	CBF-Post-BTx	CBV- Pre-BTx	CBV-Post-BTx
7. (7), F, 8y, SS, no Hb Stroke, anterior TIAs, H, L Blood Tx 08/03/2003	Pre-BTx 06/03/2003 Post-BTx 13/03/2003	*Right hemisp.* R.Sup F/P C Rinf.F/P/O C/SC R.T C/SC R.Inf.T C/SC R.inf T C *L.Hemisp.* L. T SC (BWM)	+++ +++ +++ ++ ++ =	++ ++ Unchanged +++ +++ +++	- - - - - - - - - - - - - =	Unchanged Unchanged - -/- - - Unchanged - - - -/- -	- -/- - = - - - =	=/- =/- -/- - Unchanged - - Unchanged
8. (9), M, 17 y, SS, 10.1 Stroke, H, decreased visual acuity. Blood Tx 16/03/2002	Pre-BTx 14/03/2002 Post-BTx 21/03/2002	*Right hemisp.* R.Sup P/O C/SC R.Inf.P/O C R.T/O Bdz C/SC *Left hemisp.* L.Sup.P/O C	= = + ++	Unchanged Unchanged Unchanged +++	- - - - - - - - -	Unchanged Unchanged Unchanged Unchanged	- - - - - - - -	- - - Unchanged Unchanged Unchanged

Table 6. Pre- and post-blood transfusion change in perfusion MRI parameters (mean transit time [MTT], cerebral blood flow [CBF] and cerebral blood volume [CBV]) in sickle cell patients.

Column 1: *HbSS*= homozygous sickle cell disease (sickle cell anaemia); *Hb*= haemoglobin; *TIA*= transient ischaemic attack; and *BTx*= blood transfusion.

Column 3: *hemisph.*= cerebral hemisphere, *F*= frontal region; *P*= parietal region; *T*= temporal region; *O*= occipital region; *C*= cortical; *SC*= subcortical; *BWM*= bright white matter (unexplained high signal on MTT map) and *Bdz*= borderzone.

Column 2,4,5,6,7,8 and 9: The table compares the changes in the pre-blood transfusion and post-blood transfusion perfusion MRI scans of every perfusion MRI parameter. *DSC-MRI*= dynamic susceptibility contrast MRI (perfusion MRI); *MTT*= mean transit time; *CBF*= cerebral blood flow; *CBV*= cerebral blood volume; *unchang.*= unchanged; *ext.*= extended; and *diff*= different.

MTT and *CBF* were the main parameters to assess the grade of change in cerebral perfusion of every patient before and after blood transfusion. Perfusion MRI parameters were assessed following a scale of worsening perfusion abnormality caharacterised by :
=: normal
- (for CBF and CBV): decreased passage of IV Gadolinium (-: mild, --:moderate, ---: severe)
+(for MTT): increased the mean transit time of the passage of IV Gd (+: mild, ++: moderate, +++ severe)
+ (for CBF/CBV): increased the passage of IV Gd (increased blow flow; +: mild, ++: moderate, +++: severe)
Based on the equation: *MTT= CBV/ CBF*. A significant change of the perfusion MRI parameters was defined as the improvement or worsening of the parameter (MTT, CBF, CBV) in two grades of the scale (i.e. from '= to - -'; or '+++ to +'), to minimise the effects at bias of perfusion gradation in the visual assessment of the perfusion maps of MTT, CBF and CBV by different examiners.


Worsening of perfusion MRI parameters (MTT, CBF, CBV)


Improvement (better) of perfusion MRI parameters (MTT, CBF, CBV)

Tables 7. Effect of Blood transfusion therapy on perfusion abnormality in the long-term. Patients of this study are also included in the longitudinal study (Appendix, table 3).

Case (Case in table 3), Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
1. (1) HbSS **Coma, stroke** *Coma (Posterior Leukoencepha-lopathy)* BTx	I: 30/11/1999 (9 y) F: 11/07/2002 (12 y) I: SVD F: Sum. Param. TTP similar to MTT MPC similar to CBF **Worse (CBF)**	Right Hemisp. R.P. C. ***R. P.SC.*** ***R. P/O Bdz*** Left Hemisp. ***L.P SC.***	= ++ = ++	Unchanged Unchanged Unchanged Unchanged	= = = =	- - - - - - -	= + = +	No parameter
2.(2) HbSS **Coma, stroke** *Headaches* BTx	I: 25/08/1999 (13 y) F: 15/08/2001 (16 y) **Better (CBF & CBV)**	Right hemisp. R. F C/SC R. P C/SC R. T C/SC ***R. T/O Bdz C/SC*** Left Hemisp	+++ +++ = = =	Unchanged Unchanged Unchanged Unchanged =	- - - - - - - - - - - =	Unchanged Unchanged - - - = =	- - - - - - - - - - =	Unchanged Unchanged - - - = =
3. (3) HbSS **Coma, posterior TIA** *Headaches* BTx stopped	I: 24/03/1999 (18 y) F: 19/06/2001 (19 y) **Unchanged** (WM affected?)	Right Hemisp. R.T. SC (WM) Left Hemisp. L.P. SC (WM) L.T. SC (WM)	+++ +++ +++	+++: >extended +++: >extended +++: >extended	= = =	Unchanged =/- Unchanged	= = =	Unchanged Unchanged Unchanged

Case (Case in table 3), Symptom at Presentation *Main Recurrent Symptom,* Treatment	MRI-DSC Date (age)	Perfusion Abnormality	MTT- Initial	MTT- Final	CBF- Initial	CBF- Final	CBV- Initial	CBV- Final
4. (4) HbSS **Stroke** *Seizures* BTx.	I: 12/09/2002 (6 y) F: 12/06/2003 (7 y) **Better** **(MTT & CBF)**	Right hemisp. ***R. Post P. C*** R. Sup F/P/T SC Left Hemisp. L.P. C/SC L. Sup. F/P/T C/SC L T. C/SC ***L T/O Bdz C/SC***	+++ ++ +++ +++ +++ +++	= +++ = Unchanged (less extended) Unchanged (less extended) =	- - - - - - - - - - - - - - - -	Unchanged Unchanged - - - - - - -/- - - =	-/= = -/= - = =	- - - Unchanged - - Unchanged
5-(5) HbSS **Stroke** *Headaches* BTx	I: 18/12/1998 (12 y) F: 12/10/2001 (16 y) **Worse** **(MTT &CBF)** Improved CBV	Left Hemisp. ***L Frontal C/ SC*** ***L Temporal C*** ***L F/T Bdz C/SC*** R hemisphere	++ +++ = =	+++ Unchanged +++ Unchanged	- - - - - - = =	Unchanged Unchanged - - - Unchanged	- - - - - = =	= = = Unchanged
6. (6) HbSS **Stroke** *Anterior TIA* Btx	I: 29/06/1999 (12 y) F: 17/06/2002 (16 y) **Worse** **(MTT,CBF, CBV)**	Left Hemisp. ***L.F. C/SC*** L P. C L T. C/SC Right hemisp ***R. F. C***	= ++ = =	+++ Unchanged + +++	- - - = =	- - - - - - - - - -	- - = =	- - - = - - - -

Case (Case in table 3), Symptom at Presentation *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
7. (7) HbSS **Stroke** *Anterior TIA* BTx	I:06/12/2001 (7 y) F: 06/03/2003 (8 y) **Better (MTT & CBF)**	Right Hemisp. R.F.C R.P. C. R. P. C/SC ***R.F/P Bdz SC*** R.T C/SC Left Hemisp.	++ +++ +++ +++ +++ =	+++ Unchanged Unchanged = Unchanged Unchanged	- - - - - - - - - - - - - =	Unchanged Unchanged Unchanged = - -/- - - Unchanged	- = = - =/- =	-/- - - -/- - = Unchanged Unchanged
8. (8) HbSS **Stroke** *Stroke* BTx (stopped)	I: 18/06/1999 (21 y) F: 15/11/2001 (24 y) **Worse (MTT, CBF & CBV)**	L. Hemisph L. T. SC R. Hemisph R. P. C. R. P. SC. ***R. T. C.*** R. T. SC.	? (different slice orientation) ++ +++ = +++	++ +++ Unchanged ++ ++	? (different slice orientation) - - - - - - = - - -	- - Unchanged Unchanged - - - -	? (different slice orientation) - - - - - - = - - -	- = = ++ -
9. (9) HbSS **Stroke** *Headaches* BTx	I: 17/09/1999 (14 y) F: 14/03/2002 (16 y) **Worse (MTT)**	Right Hemisp. R. P C. R. O. C. Left Hemisp. *L. O.C.*	= = =	Unchanged Unchanged ++	= - - - -	- - - - - - Unchanged	- - - - - -	Unchanged - - - Unchanged

Case (Case in table 3), Symptom at Presentation, *Main Recurrent Symptom*, Treatment	MRI-DSC Date (age)	Perfusion Abnormality	MTT- Initial	MTT- Final	CBF- Initial	CBF- Final	CBV- Initial	CBV- Final
10. (10) HbSS **Stroke** **Anterior TIA** BTx- Stopped BMT	I: 02/07/1999 (10 y) F: 20/12/2002 (13 y) **Worse** **(MTT & CBF)** Improved CBV	Right Hemisp. ***R.P. C*** ***R. P SC*** ***R.P/O Bdz C/SC*** R.T C. R.T SC. (WM?) ***R.T/O Bdz C/SC*** Left Hemisp. L.P C. ***L.P SC*** L. P/O C. ***L.T/O Bdz C***	 = = = = +++ = = = = =	 +++ +++ Unchanged + Unchanged + Unchanged +++ Unchanged +++	 - - - = - - - = - = - - - = - - =	 Unchanged - - - - - - - - - - - Unchanged - - - - - - - -	 - - - - - - - - - = = = - - - = = =	 Unchanged - - - - - Unchanged - - - -
11. (11) HbSS **Anterior TIA** *Asymptomatic* BTx	I: 13/12/2001 (15 y) F: 25/07/2003 (16 y) **Unchanged**	Right Hemisp. R. Sup. P. C Left Hemisphere	? (diff. slice orientation) =	+/++ Unchanged	? (diff. slice orientation) =	- - Unchanged	? (diff. slice orientation) =	- Unchanged

Case (Case in table 3), Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
12. (12) HbSS **Anterior TIA** *Anterior TIA* BTx- stopped	I: 23/11/1999 (21 y) F: 07/12/2001 (25 y) **Unchanged**	Right hemisp. R.F C/SC R. P SC. (WM) R. T SC (WM) Left hemisp. L. P C L. P SC (WM) L.T SC (WM)	 ? (orientation) +++ ++ +++ +++ +++	 ++ Unchanged +++ ? (orientation) Unchanged Unchanged	 ? (orientarion) - - - =/- - - - - -/- - - =/-	 - Unchanged - ? (orientation) - - - - -	 ? (orientation) - - =/- -/= - - =/-	 = Unchanged - ? (orientation) Unchanged -
13- (13) HbSS **Anterior TIA** *Anterior TIA* BTx- AED	I: 30/11/1999 (8 y) F: 04/01/2002 (11 y) **Worse (MTT & CBF)** Improvement CBV	Right Hemisp. ***R. F. C/SC*** ***R. P. C/SC*** ***R. T. C*** RT/O Bdz C/SC R.T. SC L hemisp.	 = = = ++ ++ =	 ++ ++ +++ +++ Unchanged Unchanged	 ++ ++ +++ - - =	 - - - Unchanged - - Unchanged Unchanged	 ++ ++ ++ = = =	 = + +++ + Unchanged Unchanged

Case (Case in table 3), Symptom at Presentation, *Main Recurrent Symptom*, Treatment	MRI-DSC Date (age)	Perfusion Abnormality	MTT- Initial	MTT- Final	CBF- Initial	CBF- Final	CBV- Initial	CBV- Final
14. (14) HbSS **Anterior TIA** *Anterior TIA* BTx-stopped Left indirect frontal revascularisation Hydroxyurea	I: 18/06/1999 (12 y) F: 27/08/2002 (15 y) **Better (MTT, CBF & CBV)** Worsening only in one region MTT and CBF, and another region CBV.	Right Hemisp ***R. P C*** R. P SC (WM) R. P C/SC ***R.P/O Bdz*** R. T/O Bdz R.O C Left Hemisp L. P C L. P SC (WM) ***L. P C/SC*** ***L. P/O Bdz*** L. T SC (WM) L.T/O Bdz C/SC ***L. O C***	= +++ = ++ + + Diff. orientation +++ = ++ +++ ++ ++	Unchanged Unchanged Unchanged = = = +++ ++ +++ = ++ ++ =	- - = = - - - Diff.orientation = = - -/= - - - -	= Unchanged Unchanged = = = Unchanged =/- - - = - - Unchanged =	-/- - = = = - - Diff. orientation = = = = - - - -	= Unchanged Unchanged Unchanged = = =/+ =/- =/+ Unchanged - - Unchanged =
15.(15) HbSS **Posterior TIA** *Headaches* BTx- stopped Hydroxyurea	I: 30/07/1999 (15 y) F: 04/02/2002 (19 y) **Worse (MTT, slightly CBF)**	Right Hemisp. ***R.T. C*** L. hemisphere	= =	+++ Unchanged	= =	- Unchanged	= =	Unchanged Unchanged

Case (Case in table 3), Symptom at Presentation, *Main Recurrent Symptom,* **Treatment**	**MRI-DSC Date (age)**	**Perfusion Abnormality**	**MTT- Initial**	**MTT- Final**	**CBF- Initial**	**CBF- Final**	**CBV- Initial**	**CBV- Final**
16. (16) HbSS **Posterior TIA** *Posterior TIA* BTx- stopped	I: 30/07/1999 (11 y) F: 12/11/2001 (13 y) **Better (MTT)**	Right Hemisp. ***R.T. C*** Left Hemisp.	++ =	= Unchanged	=/- =	= Unchanged	- =	= Unchanged
17 (20). HbSS **Headaches** *Headaches* BTx-stopped	I: 24/02/1999 (12 y) F: 29/08/2002 (15 y) **Better (MTT & CBF)** Worsening of post. T region (CBV) after stopped BTx	Right Hemisp. R. F C/SC ***R.T C/SC*** ***R.T/O Bz C/SC*** R.T/O Bdz C Left Hemisp ***L. T/O Bdz*** ***L.O C.***	+++ +++ +++ +++ +++ ++	++ = = Unchanged ++ +++	- - - - - - - - - - - - - - - - -	-/- - = = - -/- - - - - -/- - -	- - - - - =	- - = = - - = - -

Table 7. Long-term blood transfusion change in perfusion MRI parameters (mean transit time [MTT], cerebral blood flow [CBF] and cerebral blood volume [CBV]) in sickle cell patients.

Column 1: *HbSS*= homozygous sickle cell disease (sickle cell anaemia); *Hb*= haemoglobin; *TIA*= transient ischaemic attack; and *BTx*= blood transfusion.

Column 3: *hemisph.*= cerebral hemisphere, *F*= frontal region; *P*= parietal region; *T*= temporal region; *O*= occipital region; *C*= cortical; *SC*= subcortical; *BWM*= bright white matter (unexplained high signal on MTT map) and *Bdz*= borderzone.

Column 2,4,5,6,7,8 and 9: The table compares the changes in the pre-blood transfusion and post-blood transfusion perfusion MRI scans of every perfusion MRI parameter. *DSC-MRI*= dynamic susceptibility contrast MRI (perfusion MRI); *MTT*= mean transit time; *CBF*= cerebral blood flow; *CBV*= cerebral blood volume; *unchang.*= unchanged; *ext.*= extended; and *diff*= different.

MTT and *CBF* were the main parameters to assess the grade of change in cerebral perfusion of every patient before and after blood transfusion. Perfusion MRI parameters were assessed following a scale of worsening perfusion abnormality caharacterised by :
=: normal
- (for CBF and CBV): decreased passage of IV Gadolinium (-: mild, --:moderate, ---: severe)
+(for MTT): increased the mean transit time of the passage of IV Gd (+: mild, ++: moderate, +++ severe)
+ (for CBF/CBV): increased the passage of IV Gd (increased blow flow; +: mild, ++: moderate, +++: severe)
Based on the equation: *MTT= CBV/ CBF*. A significant change of the perfusion MRI parameters was defined as the improvement or worsening of the parameter (MTT, CBF, CBV) in two grades of the scale (i.e. from '= to - -'; or '+++ to +'), to minimise the effects at bias of perfusion gradation in the visual assessment of the perfusion maps of MTT, CBF and CBV by different examiners.

Worsening of perfusion MRI parameters (MTT, CBF, CBV)

Improvement (better) of perfusion MRI parameters (MTT, CBF, CBV)